JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXI WASHINGTON, D. C., AUGUST 15, 1921 NO. 10

A FUNGUS DISEASE SUPPRESSING EXPRESSION OF AWNS IN A WHEAT-SPELT HYBRID

By LLOYD E. THATCHER
Assistant Agronomist, Ohio Agricultural Experiment Station

During the winter of 1919, a number of F_2 hybrids between *Triticum vulgare* Vill. and *Triticum spelta* L. were grown in the greenhouse of the Department of Agronomy of the Ohio Agricultural Experiment Station. One bed was planted with 124 grains spaced about 8 inches apart each way. The seeds were first treated with the spores of *Tilletia foetans* (B. and C.) Trel., the object of the treatment being to isolate any segregates resistant to the smut that may have come out in the F_2 plants. That the treatment was successful is shown by the control.

An examination of the mature plants showed that they might be divided into three classes: Those of which all the grains of all the spikes were infected; those of which all the grains of part of the spikes were infected; and those of which all the grains of all the spikes were free from infection. The number of mature spikes varied from 1 to 6. Assuming a simple Mendelian ratio in which AA would represent the genotype of the all diseased, Aa the part diseased, and aa the disease-free, we would have the following classification:

Class.	Observed number.	Expected number.	Standard deviation.	Actual deviation.	Ratio.	
					Observed.	Expected.
All spikes diseased, AA.....	19	31	4.82	12	0.613	1
Not all spikes diseased, Aa.....	72	62	5.57	10	2.322	2
Total AA and Aa.....	91	93	4.82	2	2.935	3
All spikes disease-free, aa.....	33	31	4.82	2	1.065	1
Total AA, Aa, and aa....	124	124	.....	.....	4.000	4

It is observed that the class of the all diseased spikes is under the expectation and the class not all diseased is over the expectation. We may assume that the identification is at fault, AA sometimes giving

the same result as Aa. If the classes AA and Aa be taken as one, the deviation from expectancy is two, which is less than one-half the standard deviation.

An examination of the diseased plants revealed an interesting phenomenon as illustrated by Plate 135. Those plants which were awned segregates of the class in which part of the spikes were diseased showed normal development of the awns in the disease-free spikes and a suppression of awns in the diseased spikes. No spikes were found which contained both diseased and disease-free kernels.

PLATE 135

Spikes from three plants of F_2 wheat-spelt hybrid. The smutted spikes are on the left and the smut-free spikes on the right in each group.

EFFECT OF AMMONIUM SULPHATE UPON PLANTS IN NUTRIENT SOLUTIONS SUPPLIED WITH FERRIC PHOSPHATE AND FERROUS SULPHATE AS SOURCES OF IRON

By LINUS H. JONES and JOHN W. SHIVE
New Jersey Agricultural Experiment Station

INTRODUCTION

The experimental work reported in this paper grew out of a study of ammonium sulphate [$(NH_4)_2SO_4$] as a possible source of nitrogen for wheat and soybean plants in nutrient solutions. The status of the problem regarding the direct utilization of ammonium salts by agricultural plants is still in a very unsatisfactory condition. While experimental evidence favors the idea that ammonium salts are good sources of nitrogen for the higher plants, it also indicates that plants differ in their ability to utilize ammonium salts. Some plants appear to require the ammonium ion as well as the nitrate ion for growth at certain stages of their development. In some recent work Espino (*3*)[1] has shown that the rice plant during the early stages requires the ammonium ion for normal growth. From a review of the more important contributions to the literature on the mineral requirements of the rice plant, this author summarizes by saying:

> There is good reason to suppose that: (1) This plant requires the same chemical elements as do other higher plants. (2) The young plants are not suited to deriving their nitrogen from nitrates but thrive very well when ammonium sulphate (or possibly other ammonium salts, such as cholride, nitrate, etc.) is supplied. (3) Older plants are able to derive their nitrogen supply from nitrates, but may be able to thrive without the nitrate ion when the ammonium ion is supplied at a proper rate.

He calls attention to the fact, however, that—

> none of these points is at all well established in a quantative way.

The more recent work of Trelease and Paulino (*19*) and that of Trelease (*20*) is of interest in this connection. The results of their studies of the rice plant in soil cultures indicate that ammonium nitrogen is much more effective than nitrate nitrogen in increasing the yields of this plant. The yields obtained per unit of molecular nitrogen were highest when the nitrogen was applied to the cultures in the form of ammonium sulphate; they were lower when supplied as ammonium nitrate, and still lower when supplied in the form of calcium nitrate [$Ca(NO_3)_2$] or sodium nitrate ($NaNO_3$).

A review of the literature on the general subject of the utilization of ammonium as a source of nitrogen for the higher plants can not here be

[1] Reference is made by number (italic) to "Literature cited," p. 727-728.

attempted. Mention should be made, however, of the important work of Hutchinson and Miller (*11*) in which they summarize a review of the more definite contributions to the literature on this broad subject. As a result of their own careful research on the direct assimilation of ammonium salts by higher plants, these authors conclude that agricultural plants can develop normally when supplied with nitrogen only in the form of ammonium salts, that some plants grow equally well with ammonium or nitrate as a source of nitrogen, and others, while capable of assimilating ammoniacal nitrogen, appear better able to utilize nitrates. They express the doubt, however—

whether ammonium salts can ever produce better final results than nitrates.

In some preliminary experiments with nutrient solutions of the Tottingham (*18*) type in comparison with these solutions in which the potassium nitrate (KNO_3) was replaced by ammonium sulphate in equivalent osmotic concentrations, it was found that the solutions containing ammonium sulphate were toxic to wheat and soybean plants when soluble ferrous sulphate ($FeSO_4$) in quantities of 1 mgm. of iron per liter of nutrient solution was added as a source of iron for the plants. When, however, equivalent amounts of iron in the form of the insoluble ferric phosphate ($FePO_4$) were added to the solutions containing ammonium sulphate, the toxic effect upon the plants entirely disappeared. The phenomena observed in connection with the use of the two forms of iron (soluble and insoluble) in the presence of the ammonium ion in complete nutrient solutions appeared to be of sufficient importance to warrant careful investigation. A comparative study was, therefore, made of the physiological effects produced upon wheat and soybean plants during the early stages of development by the two types of nutrient solutions just mentioned—the Tottingham solutions unmodified and these solutions in which ammonium sulphate was substituted for the potassium nitrate—with traces of a soluble ferrous compound and an insoluble ferric compound added as sources of iron for the plants. A study was also made of the influence of the plants upon the media in which they were grown. The results obtained with wheat will alone be presented in the present paper. Those for soybeans will appear in a later publication.

METHODS OF PROCEDURE

The experiments considered in the following pages each comprised two series of cultures. In the first of these two series, 20 representative solutions chosen from Tottingham's (*18*) complete series of 84 were used. The 20 solutions chosen are uniformly distributed throughout the series and will be designated by the culture numbers referring to the positions which they occupy in the series and on the 4-coordinate diagrammatic scheme employed by Tottingham. The second series was like the first in every respect, except that ammonium sulphate in equal osmotic con-

centrations was substituted for the potassium nitrate in the Tottingham solutions of the first series. With this substitution it is reasonable to suppose that any marked differences in the response of the plants toward the nutrient media in corresponding cultures of the two series could be attributed to the influence of the ammonium sulphate upon the plants either directly or indirectly, assuming the cultures to be subjected alike to all other experimental conditions. Two control solutions serving as standards for comparison were added to each series. These consisted of Tottingham's (*18*) best solution for wheat, number $T_3R_1C_4$, with a total osmotic concentration value of 2.5 atmospheres, and Shive's (*15*) best solution for wheat, number R_5C_2, with an osmotic concentration value of 1.75 atmospheres.

Baker's analyzed salts were used in the preparation of the solutions. Table I gives the culture numbers indicating the position of the cultures in the Tottingham series and the partial volume-molecular concentrations of the salts as they occurred in the solutions of the two series. The nutrient solutions of each series were made up from half-molecular stock solutions of the salts used to give to each solution a calculated total osmotic concentration value of 1 atmosphere. Freezing-point determinations showed that this concentration was closely approximated for each solution.

TABLE I.—*Description of solutions used*

Solution No.	Volume-molecular partial concentrations.[a]				
	Tottingham series (A).	Ammonium-sulphate series (B).			
	KNO_3.	KH_2PO_4.	$Ca(NO_3)_2$.	$MgSO_4$.	$(NH_4)_2SO_4$.
$T_1R_1C_1$	0.0020	0.00211	0.00146	0.01659	0.0014
C_3	.0020	.00211	.00438	.01185	.0014
C_5	.0020	.00211	.00730	.00711	.0014
C_7	.0020	.00211	.01022	.00237	.0014
R_3C_1	.0060	.00211	.00146	.01185	.0042
C_3	.0060	.00211	.00438	.00711	.0042
C_5	.0060	.00211	.00730	.00237	.0042
R_5C_1	.0100	.00211	.00146	.00711	.0070
C_3	.0100	.00211	.00438	.00237	.0070
R_7C_1	.0140	.00211	.00146	.00237	.0098
$T_3R_1C_1$	.0020	.00633	.00146	.01185	.0014
C_3	.0020	.00633	.00438	.00711	.0014
C_5	.0020	.00633	.00730	.00237	.0014
R_3C_1	.0060	.00633	.00146	.00711	.0042
C_3	.0060	.00633	.00438	.00237	.0042
R_5C_1	.0100	.00633	.00146	.00237	.0070
$T_5R_1C_1$	.0020	.01055	.00146	.00711	.0014
C_3	.0020	.01055	.00438	.00237	.0014
R_3C_1	.0060	.01055	.00146	.00237	.0042
$T_7R_1C_1$	.0020	.01477	.00146	.00237	.0014

[a] Osmotic concentration value of each solution 1 atmosphere.

Iron was supplied to all the cultures in equivalent amounts either in the form of the so-called insoluble ferric phosphate or the soluble ferrous sulphate. These two forms of iron were chosen in order to test the effect of a soluble and an insoluble form of iron upon growing plants in connection with the two types of nutrient solutions used, and still not introduce anions different from those provided by the four main salts present in the solutions in considerable quantities. The ferric phosphate was added to the solutions from a stock supply prepared as described in a previous publication (*12*), while the ferrous sulphate was added in the form of an aqueous solution freshly prepared each time just before being used in the culture solutions. This form of iron does not precipitate so rapidly nor so completely from the culture solutions here used as do other forms of soluble iron. By direct qualitative tests made from four to seven days after the ferrous sulphate was added to the culture solutions, the presence of dissolved iron could still be detected. As indicated by these qualitative tests, the capacity of iron citrate for maintaining its solubility in these solutions was equally as strong as that of ferrous sulphate. This is in accord with the view of Hoagland (*10*), who states that iron citrate and tartrate appear to be the most efficient sources of iron. The availability and efficiency of a given iron salt in relation to plant growth are, of course, determined largely by the concentration of the iron salt used and by the concentration, reaction, and composition of the nutrient solution in which it is used, as Gile and Carrero (*5*) have shown. Duggar (*2*) very successfully used what he calls "soluble ferric phosphate," which apparenlty consists of ferric phosphate with sodium citrate, as a single salt. He states that this salt combination possesses the advantage of solubility to a high degree and introduces no difficulties in the preparation of the solutions but may render their composition somewhat less definite.

The plants used as indicators consisted of spring wheat of the Marquis variety. The seeds were germinated on a germinating net like that used by Shive (*15*). All the seedlings used were carefully selected for uniformity of size and general appearance as to health and vigor and were transferred to the culture solutions when about 5 cm. tall. Three seedlings were comprised in each culture. The seedlings were mounted in the double-piece paraffined cork stoppers as devised by Tottingham (*18*). These were of the proper size to fit the quart fruit jars which were used as containers. To exclude light from the roots and to prevent heat absorption when the cultures were exposed to sunlight the containers were covered with cylindrical shells black within and light in color on the outside like those described by Shive (*15*).

The solutions were renewed regularly at intervals of 3½ to 4 days. At the time of each renewal of the solutions tests of the hydrogen-ion concentration of each of the old solutions was made by the colorimetric

method, using the standard buffer mixtures and indicators recommended by Clark and Lubs (*1*), and these concentrations were recorded in terms of P_H values.

The plants were grown in the culture solutions during a period of five weeks. At the end of the growth periods of the different experiments the dry weights of tops and roots of each culture were obtained separately by the usual method.

In order to obtain information regarding the nonsolution environment of the plants daily records were kept, in so far as this was possible, of the measurements characterizing the aerial conditions. These data are briefly summarized in Table II, and in this form they provide some fragmentary evidence as to the sort of aerial conditions which prevailed in the greenhouse during the several experimental periods.

TABLE II.—*Maximum and minimum temperatures, average daily water loss by evaporation from standard white and black spherical atmometers, and character of days for the different experiment periods*

Experiment No.	Experimental period.		Air temperature.		Average daily evaporation.			Radio evaporation.[a]			Number of days.		
	Beginning.	Ending.	Maximum.	Minimum.	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.	Clear.	Partly cloudy.	Cloudy.
			°C.	°C.	Cc.	Cc.	Cc.	Cc.	Cc.	Cc.			
I.....	Jan. 19	Feb. 28	32.0	6.0	23.7	15.4	17.8	2.8	2.2	2.7	14	12	15
II.....	Mar. 12	Apr. 17	35.5	7.0	23.3	12.4	18.9	4.4	1.1	2.5	19	7	11

[a] The values given for radio evaporation represent the average daily excess of water loss from standard black spherical atmometer over that from the white.

EXPERIMENTAL RESULTS

For the sake of convenience in presenting the data, the two series of cultures here considered in connection with each experiment will be designated the Tottingham series (A) and the ammonium-sulphate series (B). It has already been stated that the two series as used in the different experiments are alike in every respect except that ammonium sulphate in equivalent osmotic concentrations was substituted in the solutions of the latter for the potassium nitrate in the former.

In Table III are given the dry-weight yields of tops and of roots for the cultures of the two experiments considered. All yield values are expressed in terms of the value of the first culture ($T_1R_1C_1$) in the respective series considered as unity. The absolute yield value of this culture in grams is given just below the relative value 1.00 in each case. Since the high yields are always of greater interest than are the medium or low yields, the relative dry weights of tops and of roots from the six high-yielding cultures in each series are given in the table in bold-face type.

EXPERIMENT I. FERRIC PHOSPHATE AS THE SOURCE OF IRON IN THE SOLUTIONS OF SERIES A AND OF SERIES B

The importance of the proper amounts and forms of iron to be used with a given nutrient solution in order that the plants may be adequately supplied with this element for normal growth can scarcely be overestimated. As has been shown by Gile and Carrero (*6*) and pointed out also by Hoagland (*10*), the presence in culture solutions of sufficient available iron for the development of green plants depends largely upon the form and amount of the iron salt used, upon the reaction and concentration of the nutrient solution in which it is used, and upon the time of standing. It should be added also that the different species vary considerably in their requirements for this element and in their ability to obtain it from nutrient solutions, as has been clearly brought out in preliminary experiments in connection with this study.

TABLE III.—*Relative dry-weight yields of wheat tops and roots from Tottingham's series (A) and from the ammonium-sulphate series (B) with equivalent amounts of iron supplied to each culture in the form of ferric phosphate or ferrous sulphate* [a]

Culture No.	Experiment I, source of iron $FePO_4$.				Experiment II, source of iron $FeSO_4$.			
	Series A.		Series B.		Series A.		Series B.	
	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
$T_1R_1C_1$	1.00	1.00	1.00	1.00	1.00	1.00	**1.00**	**1.00**
	(.7352)	(.1975)	(1.1316)	(.2609)	(1.0389)	(.2452)	(.9016)	(.1724)
C_3	1.35	**1.67**	**1.96**	**2.41**	**3.17**	**2.98**	.95	**1.01**
C_5	1.32	1.02	**2.05**	**2.30**	2.61	2.65	**.96**	**1.04**
C_7	.72	.91	1.10	1.53	1.39	1.79	.74	.90
R_3C_1	.92	1.11	1.26	1.11	2.04	1.15	.74	.84
C_3	.94	1.27	1.50	1.42	**3.26**	**2.98**	.81	.87
C_5	.79	.96	**1.72**	**1.61**	2.47	**3.88**	.57	.58
R_5C_1	.82	.86	.98	.92	2.55	2.12	.65	.65
C_3	.65	.96	1.12	1.23	2.83	**2.89**	.61	.58
R_7C_1	.78	1.22	.96	1.46	2.42	1.63	.69	.70
$T_3R_1C_1$	**1.44**	1.47	1.40	**1.80**	1.85	1.47	**1.16**	**1.16**
C_3	**1.40**	**1.87**	**1.68**	**2.22**	2.07	**3.26**	**1.09**	**1.10**
C_5	1.20	**1.77**	1.18	1.42	**2.90**	**3.02**	.84	.54
R_3C_1	**1.42**	1.42	**1.50**	**1.72**	**3.15**	1.63	**1.01**	.96
C_3	1.20	1.16	1.49	1.57	2.17	2.20	.76	.76
R_5C_1	1.18	1.37	1.15	1.27	**3.24**	2.77	.79	.74
$T_5R_1C_1$	**1.36**	**1.62**	**1.58**	1.15	2.37	1.92	**1.46**	**1.40**
C_3	1.31	**1.72**	1.13	1.04	1.81	2.00	.94	.97
R_3C_1	**1.61**	1.57	1.42	1.42	**2.99**	2.00	.79	.74
$T_7R_1C_1$	**1.69**	**1.87**	1.13	1.34	2.96	2.85	.94	.95
Shive's R_5C_2	1.73	2.09	.....	.....	2.39	1.84	.....	.....
Tottingham's $T_3R_1C_4$	1.65	2.33	.....	.....	2.49	3.30	.....	.....

[a] Data from the six high-yielding cultures are given in bold-face type.

Iron salts are, of course, more soluble in solutions of higher hydrogen-ion concentration than they are in solutions with lower concentrations of the hydrogen ions; but as Hoagland (*10*) has pointed out, certain forms of iron may be completely precipitated in a relatively short time when the total phosphate content of the solution is high. It appears, however, that the insoluble ferric phosphate used in the nutrient solutions here considered may be made available to the plants under certain conditions, as will be brought out by a study of the data presented in connection with these experiments.

In the first experiment each culture solution of the two series used was supplied with 0.83 mgm. of iron per liter of nutrient solution in the form of an aqueous suspension of ferric phosphate. This amount of iron was added to each culture jar at the time the solutions were renewed.

GENERAL APPEARANCE OF THE PLANTS

At the beginning of the third week of the growth period the plants in some of the cultures of the Tottingham series began to show the yellow appearance which is characteristic of plants suffering from lack of iron. This chlorotic condition later became general throughout the series. The yellowing of the leaves was usually more pronounced in the cultures having salt proportions which were unfavorable for good growth and in which the plants were smaller as a result of this unbalanced condition of the solutions. In general, however, there was good agreement in the appearance of the three plants in any one culture. That the chlorotic condition of the plants in this series was caused by the lack of available iron was made clear by the use of supplementary cultures to which iron in the form of ferrous sulphate was added in amounts equivalent to those contained in the ferric phosphate supplied to each culture. The chlorotic plants in these cultures invariably regained the normal green color of healthy plants in the course of a few days after the soluble iron was added. At the end of the growth period the plants in most of the cultures of the Tottingham series were very chlorotic and not nearly so large as were the plants in the corresponding cultures of the ammonium-sulphate series, these differences in size being paralleled by similar differences in the dry-weight yields, as an inspection of the data in Table III will show. The plants in the cultures of the ammonium-sulphate series, on the other hand, were very green; and in so far as could be judged from their general appearance they were vigorous and healthy, with the exception of the plants in several cultures which were not quite so green in color but otherwise appeared to be in good condition. The slight yellowness in the plants of these few cultures was of a different nature from that which occurred in the plants of the Tottingham series. There were, of course, large differences in the size of the plants of the different cultures resulting from the differences in salt proportions throughout the series. There was, however, no evidence of the lack of chlorophyll in the leaves, such as characterized the plants of the Tottingham series.

INFLUENCE OF THE GROWING PLANTS ON THE HYDROGEN-ION CONCENTRATION OF THE SOLUTIONS

It is, of course, well known that plants tend to change the reaction of culture solutions in which they are grown. A large number of supplementary tests made in connection with this work have brought out the fact that the rate of this change in a culture solution is dependent upon a number of different factors, some of the more important of which are

the total concentration of the solution, the relative proportions of the salt constituents, the total quantity of solution per plant, the age of the plants, and the condition of their aerial environment. From observations made during the present study the last-named factor appears to have considerable influence in determining the rate of change of the hydrogen-ion concentration of the culture solutions here used.

As previously stated, the hydrogen-ion concentrations of the culture solutions were determined at the end of the growth intervals between each two successive solution renewals throughout the entire growth period. In Table IV are presented the summarized data of these determinations in terms of P_H values. Thus, in the table are given the initial P_H values of the culture solutions, the highest and the lowest values obtained, and the average of all the values for each solution of the two series. Four days previous to harvesting the different cultures were compared with respect to yellowness of the leaves. The comparisons were made and values were obtained by means of the relative score method described by Free (*4*) for recording unmeasured plant characters. To facilitate comparisons, the results of this score are given in Table IV in connection with the hydrogen-ion exponents, the lowest score value for any culture of the two series being considered as unity.

TABLE IV.—*P_H values and score for yellowness of leaves of the culture solutions of the Tottingham series (A) and the ammonium-sulphate series (B) supplied with iron in the form of ferric phosphate*

Culture No.	Tottingham series (A).					Ammonium-sulphate series (B).				
	Score for yellowness.	P_H values.				Score for yellowness.	P_H values.			
		Initial.	Highest.	Lowest.	Average.		Initial.	Highest.	Lowest.	Average.
$T_1R_1C_1$	7.0	4.8	6.0	5.0	5.47	0.0	4.8	5.1	4.4	4.68
C_3	7.6	4.9	6.2	5.2	5.67	.0	4.8	5.4	4.2	4.75
C_5	7.7	4.8	6.2	5.1	5.70	.0	4.9	5.9	4.2	4.85
C_7	7.7	4.8	5.8	5.0	5.46	2.0	4.9	6.0	4.4	5.10
R_3C_1	7.7	4.8	6.1	5.1	5.55	.0	4.9	5.8	4.2	4.76
C_3	1.3	4.8	6.0	5.2	5.61	.0	4.9	6.0	4.1	4.75
C_5	7.7	4.9	6.0	5.2	5.55	1.0	4.9	5.0	4.1	4.39
R_5C_1	7.8	4.9	5.9	5.0	5.57	1.9	4.9	5.6	4.1	4.62
C_3	7.7	4.8	6.0	5.2	5.60	1.0	4.9	5.7	4.1	4.53
R_7C_1	6.6	4.9	6.0	5.5	5.71	2.0	4.9	5.9	4.0	4.59
$T_3R_1C_1$	4.2	4.6	5.9	4.8	5.33	.0	4.6	5.6	4.2	4.70
C_3	4.2	4.7	5.9	4.8	5.39	.0	4.6	5.2	4.4	4.69
C_5	4.2	4.8	5.9	4.9	5.33	.0	4.8	5.8	4.6	4.89
R_3C_1	4.2	4.8	5.9	5.0	5.39	.0	4.7	5.2	4.1	4.50
C_3	4.2	4.8	6.0	4.8	5.40	.0	4.8	4.9	4.1	4.47
R_5C_1	7.6	4.8	5.9	5.1	5.49	.0	4.8	5.7	4.1	4.72
$T_5R_1C_1$	4.3	4.6	5.8	4.9	5.24	.0	4.6	5.7	4.4	4.84
C_3	4.4	4.7	5.9	4.7	5.32	.0	4.6	5.6	4.2	4.72
R_3C_1	4.3	4.6	5.9	4.8	5.36	.0	4.6	5.5	4.1	4.66
$T_7R_1C_1$	4.4	4.6	5.9	4.8	5.32	1.8	4.6	5.4	4.4	4.70
Shive's— R_5C_2	1.8	4.6	5.6	4.6	4.96					
Tottingham's— $T_3R_1C_4$	4.2	4.6	5.4	4.6	4.94					

The data of Table IV show that the hydrogen-ion concentrations of the culture solutions of the Tottingham series were always considerably

decreased during any growth interval. The culture solutions of the ammonium-sulphate series, on the other hand, usually increased somewhat in hydrogen-ion concentrations. The average P_H values of the culture solutions of this series do not vary greatly from the initial values. For most of the solutions these average values were slightly lower and a few were slightly higher than were the initial P_H values of the solutions.

To bring out more clearly the magnitude and the direction of the change in reaction of the culture solutions during the different intervals throughout the growth period, detailed hydrogen-ion concentration data for the culture solutions producing the highest, medium, and lowest yields in each of the series are given in Table V. From the data of this table and those of Table IV it can be definitely stated that the effect of the growing plants was always to decrease the hydrogen-ion concentration of the culture solutions of the Tottingham series and to increase the concentration of the hydrogen ions of the solutions of the ammonium-sulphate series, or, at least, to maintain the initial concentrations of these ions during the first four weeks of the growth period.

TABLE V.—P_H *values of the culture solutions of the Tottingham series (A) and the ammonium-sulphate series (B) producing highest, medium, and lowest yields with iron supplied in the form of ferric phosphate* [a]

Dates of solution renewals and growth intervals.	Tottingham series (A) producing—			Ammonium-sulphate series (B) producing—		
	Highest yield ($T_7R_1C_1$).	Medium yield ($T_3R_1C_5$).	Lowest yield ($T_1R_5C_5$).	Highest yield ($T_1R_1C_5$).	Medium yield ($T_3R_3C_3$).	Lowest yield ($T_1R_7C_1$).
1919.						
Aug. 22	4.6	4.8	4.8	4.9	4.6	4.9
26	4.8	4.9	5.2	4.9	4.8	4.5
29	4.9	5.0	5.4	5.0	4.8	4.8
Sept. 2	5.0	5.1	5.4	4.8	4.6	4.4
5	5.1	5.1	5.3	4.6	4.6	4.4
9	5.4	5.4	5.5	4.2	4.7	4.1
12	5.3	5.3	5.5	4.6	4.6	4.2
16	5.7	5.6	5.9	4.4	4.6	4.1
19	5.5	5.6	5.8	4.2	4.4	4.0
23	5.9	5.9	6.0	5.9	5.2	5.9
27	5.6	5.4	6.0	5.9	4.6	5.5
Average	5.32	5.33	5.60	4.85	4.69	4.59

[a] The first number in each column represents the initial P_H value of the solution.

The exact manner in which the hydrogen-ion concentrations of the nutrient solutions are altered by the growing plants has not yet been clearly demonstrated. Hoagland (*8*) has suggested that the change in reaction must be the result of the secretion of neutralizing substances by the plants, of chemical reaction with the material of the roots, or of the selective absorption of specific ions. As a result of extensive studies of absorption of ions by plants, Pantanelli (*13*) ascribes the change in reaction of the nutrient media mainly to the differential absorption of the ions by the living plant roots.

It appears that the type of the culture solution as determined by the nature of ion constituents is in a large measure responsible for the direction of the change in reaction produced by the growing plants. Hoagland (*10*) in a recent publication has emphasized the point that a nutrient solution is an exceedingly complex system and that it does not appear possible at the present time to determine quantitatively the ions and undissociated salts which it may contain during or after the growth of plants in it, much less does it appear possible to determine the exact relationship existing between the different components of such a complex system. In an earlier publication (*8*) the last-named author came to the conclusion that plants may regulate the reaction of the nutrient medium in such a way that excessive hydrogen or hydroxyl ions can not occur. In later work (*9*) he found that barley plants grown for seven weeks in a favorable nutrient solution when transferred to single salt solutions of various salts did not bring about an unfavorable condition of acidity or alkalinity. In this work, however, he noted that ammonium-chlorid solutions retained an acid reaction and the hydrogen-ion concentration was slightly increased after contact with the plants. Toole and Totting-ham (*17*), working with Knop's solution, call attention to the fact that growing barley seedlings always had a marked neutralizing effect upon the medium. It is of interest here to note that plants grown in the Shive's (*15*) 3-salt solution always show a marked tendency to decrease the hydrogen-ion concentrations, but when ammonium sulphate, even in relatively small amounts, is superimposed upon this solution the direction of the change in reaction is reversed, just as it is when this salt is substituted for potassium nitrate in the Tottingham solutions. This is true of plants during at least the first four weeks of growth.

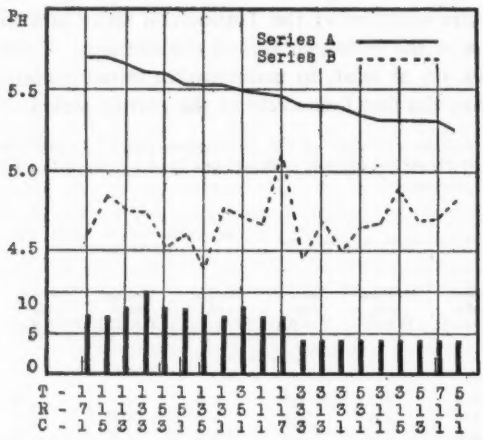


FIG. 1.—Graphs of PH values of culture solutions after contact with plant roots during growth interval between solution renewals; averages of tests during growth period of experiment I. Also diagram of score values for yellowness. Broad and narrow vertical lines represent score for plants of Tottingham series and ammonium-sulphate series, respectively.

Inspection of the data in Table IV brings out the fact that the initial P_H values of the corresponding solutions of the two series are approximately the same. The averages of the P_H values determined at the end of the various growth intervals are, however, always much higher for the

culture solutions of the Tottingham series than they are for the corresponding ones of the ammonium-sulphate series. This is brought out more clearly by the graphs of figure 1, which represent the average P_H values plotted in the descending order of their magnitudes in the Tottingham series. The relative values of the score for yellowness for the cultures of the Tottingham series and of the ammonium-sulphate series are represented graphically by broad and narrow vertical lines, respectively, just below the graphs representing the average P_H values.

Comparing now the score for yellowness of leaves with the average P_H values obtained at the end of the growth intervals, it will be observed that the higher P_H values throughout the Tottingham series correspond in a general way to high relative score values for yellowness in this series. The P_H values throughout the series decrease slightly with increase in the phosphate content of the solutions, and these slight differences are generally paralleled by somewhat more pronounced differences in the relative values of the score for yellowness.

In the cultures of the ammonium-sulphate series the plants were entirely free from any chlorotic condition such as characterized the plants in the Tottingham series the culture solutions of which had much higher average P_H values. This is also apparent from the color score, which, however, shows a slight degree of yellowness for the plants of several cultures only of the ammonium-sulphate series to which reference has previously been made.

Attention has already been called to the fact that the chlorotic condition of the plants in the Tottingham series was due to an insufficient supply of available iron, since the addition of soluble iron to the solutions of such cultures enabled the plants to regain their normal green color and to overcome completely the chlorotic condition in the course of a few days. There was no evidence, however, of the lack of available iron in the culture solutions of the ammonium-sulphate series. Thus, by the substitution of ammonium sulphate for potassium nitrate in the Tottingham solutions, the ferric phosphate here used as a source of iron for the plants was rendered available perhaps by the maintenance of a higher average concentration of the hydrogen ions during the growth of the plants in the solutions, since the solubility of iron is, of course, greater in solutions of higher hydrogen ion concentrations.

DRY-WEIGHT YIELDS OF TOPS AND ROOTS

The relative dry-weight yields of tops and of roots from the cultures of the two series here considered are given in Table III. The absolute dry-weight yield values corresponding to these relative data have been plotted to form the graphs of figures 2 and 3, in which the ordinates represent dry weights in grams and the abscissas represent the different cultures. The values are always plotted according to the descending order of their magnitudes in the Tottingham series.

The graphs of figure 2 show at once that the yields of tops from the cultures of the ammonium-sulphate series are much superior to the yields from the corresponding cultures of the Tottingham series. In general this is true also with respect to the yields of roots from the two series as is indicated by the graphs of figure 3. However, the root yields from three cultures of the ammonium-sulphate series are actually lower than are the yields from the corresponding cultures of the Tottingham series. The superiority of the yields from the ammonium-sulphate series is clearly not due to a greater efficiency of the four main salt constituents or combinations of the solutions of the series but is the direct result of the difference in the availability of the iron in the form here used in the solutions of the two series during the first five weeks of growth.

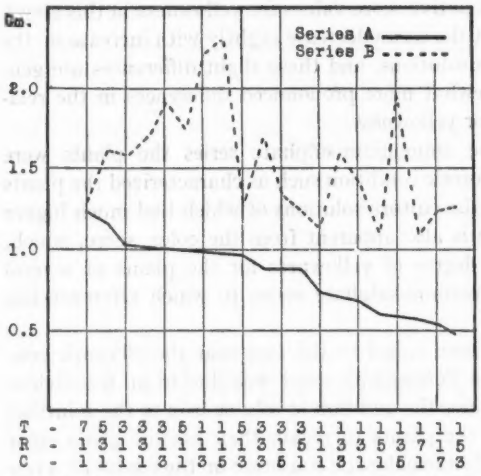


FIG. 2.—Graphs of actual yield vaules of wheat tops for the Tottingham series and the ammonium-sulphate series of Experiment I.

In the Tottingham series the growth of the plants was greatly retarded after the third week of growth by a deficiency in the supply of available iron, while no such deficiency of iron was at any time apparent in the culture solutions of the ammonium-sulphate series. The nature of the nutrient solutions with respect to their salt constituents and hydrogen-ion concentration here appears to determine the availability and the efficiency of the ferric phosphate as a source of iron for the plants. In connection with this study the work of Wolkoff (*21*) is of interest. In a sand culture procedure with soybeans this author used the same series of nutrient solutions employed in the present study, but the solutions had an osmotic concentration value of 2.5 atmospheres and iron was supplied in the form of iron rust. A marked superiority of the yields from the culture containing ammonium sulphate over those from the Tottingham solutions was shown, but the author states that the most pronounced differences in the plants from the two series of cultures were those of color, the plants from the cultures containing ammonium sulphate always being much greener than those from the Tottingham series.

EXPERIMENT II. FERROUS SULPHATE AS THE SOURCE OF IRON IN THE SOLUTIONS OF SERIES A AND SERIES B

The solution cultures used in the two series of this experiment were the same as those of the preceding experiment except that soluble ferrous sulphate was used as the source of iron for the plants instead of the insoluble ferric phosphate used in experiment I. As before 0.83 mgm. of iron per liter of nutrient solution was supplied to each culture jar at the time when the solutions were renewed. The ferrous sulphate was added in the form of an aqueous solution which was always freshly prepared just before being used. The treatment of the plants before and during the growth period, the renewal of the solutions, and the general culture methods were carried out in the same manner as were those in the preceding experiment.

GENERAL APPEARANCE OF THE PLANTS

During the first two weeks of the growth period there was no marked difference in the general appearance of the plants in the two series. The plants in the Tottingham series as a whole presented perhaps a slightly better appearance and showed a somewhat sturdier growth than did the plants in the ammonium-sulphate series. After 20 days the plants in some of the cultures of the ammonium-sulphate series showed a tendency to become weakened; the leaves drooped, presenting angles instead of curves at the weakened points, and the growth rates were greatly retarded. Later this condition became general throughout the series and increased in intensity as the plants grew older, giving them a very dull, unhealthy appearance. In a few cultures of this series another symptom of physiological disturbance appeared in the form of narrow white stripes in the leaves. This condition was quite unlike the chlorotic appearance of the leaves of plants suffering from lack of iron.

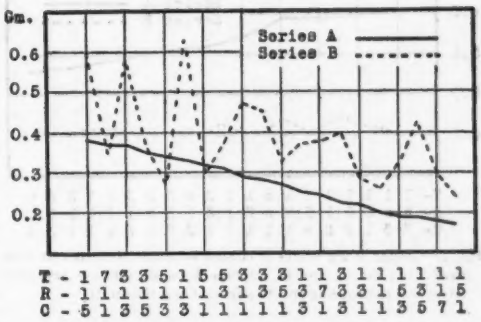


FIG. 3.—Graphs of actual yield values of wheat roots for the Tottingham series and the ammonium-sulphate series of experiment I.

The plants of the ammonium-sulphate series here presented a sharp contrast to those of the Tottingham series in which no toxic symptoms occurred at any time during the growth period. The plants in the latter series were at all times dark green in color and appeared vigorous and healthy, making rapid growth. There were, of course, large differences

in the size of the plants of the different cultures in this series as a result of variations in the salt proportions of the culture solutions, as the dry weights in Table III will show. Thus, by the use of the soluble ferrous sulphate in small quantities, instead of the insoluble ferric phosphate used in the solutions of the preceding experiment, the general health and vigor of the plants in the Tottingham series was very greatly improved, while the opposite effect was experienced by the plants in the cultures of the ammonium-sulphate series.

INFLUENCE OF THE PLANTS UPON THE HYDROGEN-ION CONCENTRATION OF THE MEDIA

The summarized data of the hydrogen-ion concentration determinations of this experiment are given in Table VI in terms of P_H values. The data of this table correspond in every respect to the similar data of Table IV. From the data of Table VI it is again evident that the growing plants in the Tottingham series showed a marked tendency to change the reaction of the solutions toward neutrality, while the direction of this change in reaction was reversed by the growing plants in the solutions of the ammonium-sulphate series. In the latter series the average of the P_H values obtained at the end of the several growth intervals was lower for each culture solution throughout the entire series than was the corresponding initial P_H value, although the increase in the hydrogen-ion concentration was not very great in any case.

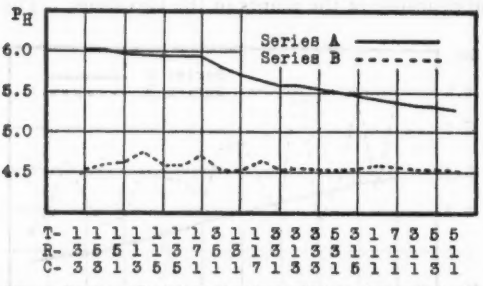


FIG. 4.—Graphs of P_H values of culture solutions after contact with plant roots during growth interval between solution renewals; averages of tests during growth period of experiment II.

The averages of the P_H values determined at the end of the various growth intervals are always much higher for the cultures of the Tottingham series than they are for the corresponding cultures of the ammonium-sulphate series. This is clearly brought out by the graphs of figure 4. A direct comparison of these graphs with the corresponding ones of the preceding experiment (fig. 1) shows that the two sets of graphs are generally in good agreement. This indicates that the influence of the growing plants upon the reaction of the culture solutions here used was practically the same when the iron was supplied in the form of the insoluble ferric phosphate or in the form of the soluble ferrous sulphate.

The detailed hydrogen-ion concentration data for the culture solutions producing highest, medium, and lowest yield given in Table VII show in a general way the direction and the magnitude of the change in

reaction in these solutions produced by the plants during the various intervals throughout the growth period. The data of this table, which agree in a general way with the corresponding data of Table V, are fairly representative of similar data for all the cultures of the respective series in this experiment. They further emphasize the point already brought out, that the different forms of iron here used have little if any effect upon the plants with respect to their influence upon the hydrogen-ion concentration of the nutrient media, except in so far as differences in size and vigor of the plants or differences in the extent of the absorbing surfaces of the root systems may produce somewhat corresponding differences in the magnitudes of the changes in reaction. On the other hand, the different forms of iron used in either of the two types of solutions here used produced markedly different effects upon the general appearance, health, and vigor of the plants and upon the growth rates, as will be more fully brought out by a further consideration of the dry-weight yields of tops and roots.

TABLE VI.—P_H *values of the culture solutions of the Tottingham series (A) and the ammonium-sulphate series (B) supplied with iron in the form of ferrous sulphate*

Culture No.	Tottingham series (A).				Ammonium-sulphate series (B).			
	Initial.	Highest.	Lowest.	Average.	Initial.	Highest.	Lowest.	Average.
$T_1R_1C_1$	4.8	6.0	4.8	5.42	4.8	4.7	4.4	4.59
C_3	4.8	6.6	5.0	5.97	4.8	5.0	4.5	4.74
C_5	4.8	6.6	5.0	5.96	4.8	4.7	4.4	4.60
C_7	4.8	6.3	4.9	5.65	4.9	4.8	4.4	4.63
R_3C_1	4.8	6.3	4.9	5.72	4.8	4.9	4.4	4.53
C_3	4.8	6.6	5.0	6.03	4.9	4.6	4.4	4.53
C_5	4.8	6.6	5.0	5.96	4.8	4.7	4.4	4.60
R_5C_1	4.8	6.6	4.9	5.98	4.9	4.7	4.4	4.61
C_3	4.8	6.6	4.9	6.03	4.9	4.8	4.4	4.59
R_7C_1	4.8	6.6	5.0	5.96	4.9	5.1	4.4	4.71
$T_3R_1C_1$	4.7	5.9	4.6	5.33	4.7	4.7	4.4	4.55
C_3	4.6	6.3	4.6	5.59	4.7	4.7	4.4	4.56
C_5	4.7	6.0	4.7	5.48	4.7	4.7	4.3	4.55
R_3C_1	4.7	6.3	4.7	5.60	4.7	4.7	4.4	4.53
C_3	4.7	6.3	4.6	5.54	4.7	4.6	4.4	4.54
R_5C_1	4.7	6.5	4.9	5.81	4.7	4.6	4.4	4.54
$T_5R_1C_1$	4.6	5.9	4.5	5.28	4.7	4.6	4.4	4.54
C_3	4.6	5.8	4.6	5.32	4.6	4.7	4.4	4.56
R_3C_1	4.7	6.2	4.6	5.50	4.6	4.6	4.4	4.54
$T_7R_1C_1$	4.6	5.9	4.5	5.37	4.6	4.7	4.4	4.56
Shive's R_5C_2	4.6	5.6	4.6	5.09	.....	.....	.....	.....
Tottingham's $T_3R_1C_4$	4.6	5.5	4.6	5.06	.....	.....	.....	.....

TABLE VIII.—P_H *values of the culture solutions of the Tottingham series (A) and the ammonium-sulphate series (B) producing highest, medium, and lowest yields with iron supplied in the form of ferrous sulphate*[a]

Dates of solution renewals and growth intervals.	Tottingham series (A) producing—			Ammonium-sulphate series (B) producing—		
	Highest yield ($T_1R_3C_3$).	Medium yield ($T_3R_3C_3$).	Lowest yield ($T_1R_1C_1$).	Highest yield ($T_5R_1C_1$).	Medium yield ($T_3R_3C_1$).	Lowest yield ($T_1R_3C_5$).
1920.						
Mar. 12	4.8	4.7	4.8	4.7	4.7	4.8
16	5.0	4.6	4.8	4.4	4.4	4.6
19	5.3	4.9	5.1	4.6	4.4	4.5
23	5.6	5.2	5.2	4.5	4.5	4.5
26	5.9	5.3	5.3	4.4	4.4	4.4
30	6.3	5.6	5.5	4.6	4.5	4.7
Apr. 2	6.3	5.7	5.4	4.6	4.7	4.6
6	6.3	5.6	5.3	4.6	4.6	4.7
9	6.5	5.9	5.7	4.6	4.6	4.6
13	6.6	6.3	5.9	4.6	4.6	4.7
17	6.5	6.3	6.0	4.6	4.6	4.7
Average	6.03	5.54	5.42	4.54	4.53	4.60

[a] The first number in each column represents the initial P_H value of the solution.

DRY WEIGHTS OF TOPS AND ROOTS

The relative yield values for the cultures of the two series of this experiment are given in Table III in connection with the relative dry-weight yields from the cultures of the preceding experiment. The absolute dry weights of tops and roots were plotted to form the graphs of figures 5 and 6, respectively. These graphs were prepared in the same manner as those representing the dry-weight yields in figures 2 and 3. From the graphs of figures 5 and 6 it will be observed that the yields of both tops and roots from the cultures of the Tottingham series were always much superior to those from the corresponding cultures of the ammonium-sulphate series, in which only a single culture gave a dry-weight yield of tops which was higher than the lowest yield of tops in the Tottingham series. In the ammonium-sulphate series all the root yields were lower than the lowest yield in the Tottingham series.

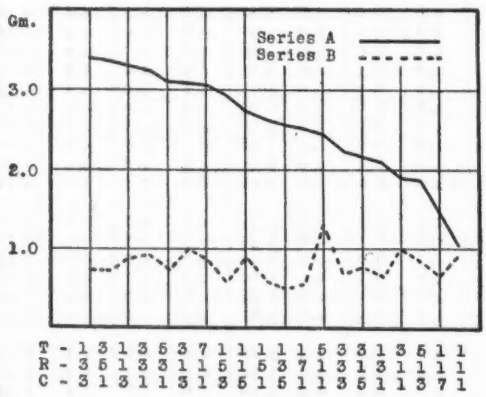


FIG. 5.—Graphs of actual yield values of wheat tops for the Tottingham series and the ammonium-sulphate series of experiment II.

Comparing now the graphs of figure 5 with those representing the dry-weight yields of tops from the two series of the preceding experiment (fig. 2), it will be observed that the relative positions of the graphs representing corresponding series of the two experiments are completely reversed. Thus, in experiment I (fig. 2) the graphs representing top yields from the cultures of the Tottingham series, throughout its entire length, lies much below the graph representing these yields from the ammonium-sulphate series, while the corresponding graphs in experiment II (fig. 5) occupy exactly opposite positions. A similar comparison of the two sets of graphs representing the dry-weight yields of roots from the two series in each experiment (fig. 3, 6) shows that these graphs have the same sort of arrangement as the graphs representing the dry weight of tops. It should be noted, however, that the differences between the yield values of tops, and especially of roots, from corresponding cultures of the two series, as indicated by the pairs of graphs representing the yields in question, are always much less pronounced in experiment I than they are in experiment II.

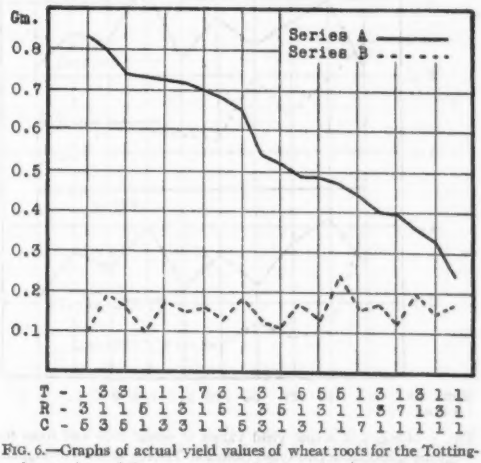


FIG. 6.—Graphs of actual yield values of wheat roots for the Tottingham series and the ammonium-sulphate series of experiment II.

The small amounts of iron used here with each culture (0.83 mgm. per liter of solution) appeared to be sufficient to prevent chlorotic effects in the plants of the Tottingham series and to provide them with the necessary supply of this element for normal growth; and, as already stated, the plants of this series were in excellent condition during the entire growth period and produced very high yields. In the solutions of the ammonium-sulphate series, on the other hand, the soluble iron in the small quantities used with each culture was very toxic to the plants, which were greatly retarded in their growth rates and produced very low yields. That this condition of the plants was directly related to the form of iron supplied is clear from the fact that in the preceding experiment these same culture solutions provided with iron, in equivalent amounts, in the form of the insoluble ferric phosphate, produced healthy, vigorous plants and high yields.

In order to test the effect of varying amounts of iron in the form of the soluble ferrous sulphate upon the growth of wheat plants in the two types of culture solutions used here, a special experiment was carried

out. This comprised two series of 10 cultures each. Throughout each series the same solutions were used for all the cultures, these solutions differing only in the amounts of iron contained. For one of these series Tottingham's solution number $T_3R_1C_5$ was chosen, and for the other this solution was modified by substituting ammonium sulphate for the potassium nitrate, as previously explained, in equivalent osmotic concentration (solution number $T_3R_1C_5$ of the ammonium-sulphate series in experiments I and II). These two series will be designated series C and series D. Iron was supplied to the solutions of each series in amounts varying from 0.01 mgm. to 5.0 mgm. The culture methods pursued with these two supplementary series were precisely the same as those described and used in carrying out the main experiments of this study. The cultures were conducted during a growth period of 35 days.

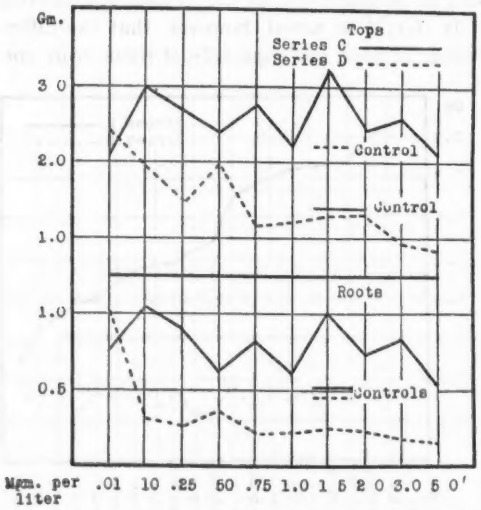


FIG. 7.—Graphs of actual yield values of wheat tops and roots for culture solutions of two types (solution $T_3R_1C_5$ of the Tottingham series and solution $T_3R_1C_5$ of the ammonium-sulphate series) supplied with varying amounts of iron in the form of ferrous sulphate. Series C and D of experiment II.

During the third week of the growth period evidences of toxicity began to appear in the plants growing in the solutions containing ammonium sulphate. The plants assumed an unhealthy, slightly yellowish, dull color, which was entirely different from the characteristic chlorotic appearance of plants suffering from the insufficient supply of iron. A weakened condition of the plants was evident from the manner in which the leaves drooped and the growth rates were retarded. The plants in the solutions containing the highest amounts of iron were the first to be affected, and the toxicity increased in severity as the plants grew older and spread to include all of the cultures except the one containing the smallest amount of iron (0.01 mgm. per liter of solution). The plants in this culture were at all times green and healthy, grew rapidly, and produced relatively high yields. The plants in the Tottingham solutions were green and healthy throughout the growth period, with the exception of those in the two solutions containing 0.01 mgm. and 0.10 mgm. of iron per liter of solution. The plants in the first of these two solutions were chlorotic, while those in

the second were only slightly chlorotic as the result of an insufficient supply of available iron.

At the end of the growth period of 35 days the dry weights of tops and roots were obtained in the usual way. The yield for the cultures of the two series, together with the average hydrogen-ion exponents of the solutions obtained at the end of the various growth intervals, are given in Table VIII. The yield values of tops and roots as given in this table are represented graphically in figure 7, the upper set of graphs representing the yields of tops and the lower set the root yields. The dry-weight values as ordinates are here plotted against the amounts of iron in milligrams per liter of solution as abscissas.

TABLE VIII.—*Hydrogen-ion concentrations and dry-weight yields of wheat grown in two types of nutrient solutions supplied with varying quantities of iron in the form of ferrous sulphate*

Quantities of iron (in milligrams per liter of solution).	Tottingham's solution ($T_3R_1C_5$), series C.			Tottingham's solution ($T_3R_1C_5$) modified, [$(NH_4)_2SO_4$ substituted for KNO_3], series D.		
	Dry weight of tops	Dry weight of roots.	Average PH values.[a]	Dry weight of tops	Dry weight of roots.	Average PH values.
	Gm.	*Gm.*		*Gm.*	*Gm.*	
0.00	1.4017	0.5113	5.53	2.2010	0.4726	4.63
.01	2.1212	.7513	5.68	2.4131	1.0250	4.54
.10	3.0448	1.1837	5.68	1.9525	.3233	4.64
.25	2.6691	.8798	5.63	1.4792	.2758	4.60
.50	2.3908	.6265	5.60	2.0002	.3712	4.64
.75	2.7478	.8182	5.59	1.1501	.2227	4.63
1.00	2.2148	.6210	5.59	1.1937	.2263	4.60
1.50	3.1924	1.1366	5.61	1.3032	.2543	4.64
2.00	2.4451	.7448	5.58	1.3113	.2354	4.63
3.00	2.5870	.7962	5.55	.9408	.1829	4.56
5.00	2 2296	.5558	5.51	.8288	.1608	4.53

[a] These values represent the average of all the determinations made for each solution at the end of the growth intervals throughout the experiment period. The initial PH values of all the solutions in each series were approximately 4.7.

As indicated by the graphs of figure 7 the yields of tops and roots from the cultures containing ammonium sulphate (series D), not including the yield from the first culture in the series, were always much inferior to the corresponding yields from the Tottingham solutions. The first three cultures show a rapid decline in yield with increasing small amounts of iron, after which the decrease in yields is less marked, although there is a general decrease in the yields of both tops and roots with increasing amounts of iron throughout the series, as the gradual downward slope of the graphs indicates. This is in entire agreement with the general appearance and condition of the plants as already described, with respect to the evidence of a toxic influence as indicated by the apparent size, color, and the generally depressed condition of the plants which was progressively more pronounced with increasing amounts of iron.

The graphs representing the yields from the Tottingham solutions (series C), on the other hand, show that in these solutions the variations in the amounts of iron within the range here used (0.01 mgm. to 5.0 mgm. per liter of solution) did not produce marked differences in the yields. All the solutions of this series produced relatively high yields, and there were no evidences of specific toxicity, although, as previously stated, the 0.01–mgm. and the 0.10–mgm. portions of iron were not sufficient to prevent chlorosis in the plants grown in these solutions.

It will be observed that the control culture (without iron) in the ammonium-sulphate series (series D) produced a much higher yield than did the control culture in the Tottingham series (series C). This agrees with the general appearance of the plants in these two cultures, the plants in the former being green and apparently healthy while those in the latter were chlorotic. The large difference in the dry-weight yields of the plants from these control cultures may possibly be accounted for by the fact that the magnesium-sulphate and monopotassium-phosphate crystals contained small amounts of iron (less than 0.001 per cent by analysis) as an impurity. This very small trace of iron was perhaps soluble in the solution containing ammonium sulphate and available to the plants and insoluble and, therefore, unavailable to the plants in the Tottingham solution. This is offered only as a suggestion and may not explain the real cause of the large difference in dry top yields, since the difference in the root yields from these control cultures is very slight.

In this connection it might be suggested that the toxic influence of the iron in the solutions containing ammonium sulphate is perhaps directly related to the hydrogen-ion concentrations of the culture solutions. The initial hydrogen-ion exponents of the solutions used in series C and D are approximately the same (P_H 4.7); but the hydrogen-ion concentrations of the Tottingham solutions change rather rapidly toward the neutral point when in contact with the plant roots, while under the same conditions the hydrogen-ion concentrations of the solutions containing ammonium sulphate are increased, or at least maintained at their initial values, during the several growth intervals between successive solution changes throughout the experiment period as is indicated by the data in Table VIII. Since iron compounds are less soluble in culture solutions with lower hydrogen-ion concentrations, it is possible that in the Tottingham solutions with decreasing concentrations of these ions sufficient iron was perhaps removed from solution by precipitation to prevent the toxic influence of this element. The suggestion here made appears to gain some support from actual observation. Precipitates invariably occurred in both the Tottingham solutions and the solutions containing ammonium sulphate, in which the proportions of iron as here used were relatively high, after standing in contact with the plant roots; but these precipitates always appeared first in the Tottingham solutions and were always much more pronounced in these than they were in the solutions containing ammonium sulphate.

Experience has, of course, shown that culture solutions may not be expected to produce good growth of green plants if they contain more than a very small quantity of available iron, and observations of toxicity produced by iron under various experimental conditions are frequent in the literature. Thatcher (*16*) makes the broad statement that—

> Only soluble ferric compounds seem to serve as a suitable source of supply of the element; ferrous compounds being usually highly toxic to plants.

In this connection, Hartwell and Pember (*7*) found ferrous sulphate to be toxic to barley and rye seedlings when added to Knop's solution in concentrations of *N/5,000*. This concentration, however, is much higher than the concentrations of ferrous sulphate used in the present experiments. These authors state that the absolute effect of a given strength of ferrous sulphate would be expected to depend upon the frequency of the renewed applications and upon the nature of the nutrient solution employed. Ruprecht (*14*) also found that ferrous sulphate when present in culture solutions above four parts per million of iron exerts a toxic effect upon clover seedlings.

From a consideration of the data presented in the preceding pages it is evident that the form and quantity of iron in a medium for plant growth is a very important factor. In experiment I of this study the iron supplied to the plants in the form of ferric phosphate was entirely inadequate to support plant growth in the Tottingham solutions. When, however, iron in this form was supplied to the plants in the solutions containing ammonium sulphate which was substituted for the potassium nitrate in the Tottingham solutions, iron did not become a limiting factor for growth and these solutions produced excellent plants which were vigorous and healthy throughout the early stages of growth extending over a period of five weeks. On the other hand, when iron was supplied in the form of the soluble ferrous sulphate, the Tottingham solutions supported excellent growth and produced very high yields of both tops and roots, while the solutions containing ammonium sulphate were exceedingly toxic to the plants, and this toxicity was intensified by the use of higher concentrations of the iron salt but disappeared entirely when only a small trace of iron (0.01 mgm. per liter) was supplied. It appears, therefore, that the injury to the plants did not result from the direct influence of the ammonium salt here used but was caused by the iron which was made available in excessive quantities through the presence of the ammonium salt.

YIELDS OF TOPS AND ROOTS IN RELATION TO SALT PROPORTIONS

The relation between yields and salt proportions will here be considered for two series only, these being the Tottingham series (A) of experiment II and the ammonium-sulphate series (B) of experiment I. In these two series the plants were at all times healthy and vigorous and suffered no apparent injury. The plants of the Tottingham series (A)

of experiment I and those of the ammonium-sulphate series (B) of experiment II were greatly restricted in the growth rates, as already explained. The yields from these two series will, therefore, not be considered in relation to the salt proportions of the culture solutions.

The relative yield values as given in Table III for the six cultures producing the highest yields in each of the two series here to be considered were plotted on the tetrahedral diagram like that employed by Tottingham (*18*) but here presented in perspective in somewhat the same manner as was done by Espino (*3*). Since the low and medium yields have little interest in this connection, they will be omitted from the diagrams and discussion. The high yields of tops for both series were plotted

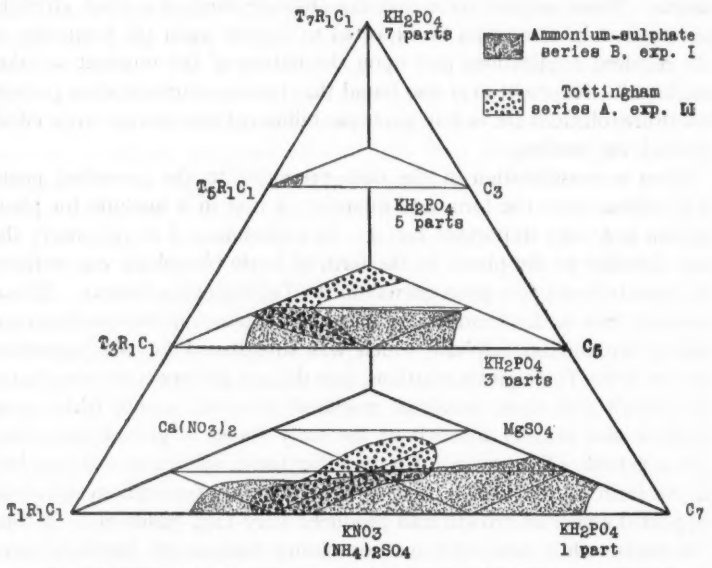


FIG. 8.—Distribution of relative dry-weight values of tops for the best six cultures in each series.

on a single diagram, as were also the corresponding yields of roots for the two series. The areas representing the high yields of the two series are distinguished by shading. On the diagram of figure 8 the dotted areas represent the yields of tops from the best six cultures of the Tottingham series (series A of experiment II), and the stippled areas represent the corresponding yields from the best six cultures of the ammonium-sulphate series (series B of experiment I). The relative dry-weight yields of roots from the best six cultures of each of the two series are similarly represented on the diagrams of figure 9.

Comparing now the areas representing the high yields of tops from the Tottingham series with those representing the corresponding yields from the ammonium-sulphate series, as shown on the diagram of figure 8, it

will be observed that there is considerable overlapping of these areas. Out of the group of six high-yielding cultures in each series three are corresponding cultures and are included in the areas marking high yields of tops in both series. These three cultures are $T_1R_1C_3$, $T_1R_3C_3$, and $T_3R_3C_1$. The maximum yield from the Tottingham series was produced by culture $T_1R_3C_3$, while the maximum yield from the ammonium-sulphate series occurred with culture $T_1R_1C_5$. A similar comparison of the areas of high root yields on the diagram of figure 9 also shows a certain amount of overlapping of the areas representing these yields from the two series under consideration. The three cultures $T_1R_1C_3$, $T_1R_3C_5$, $T_3R_1C_3$ are included in the areas representing high root yields in both series. The maximum yields of roots were produced by cultures $T_1R_3C_5$

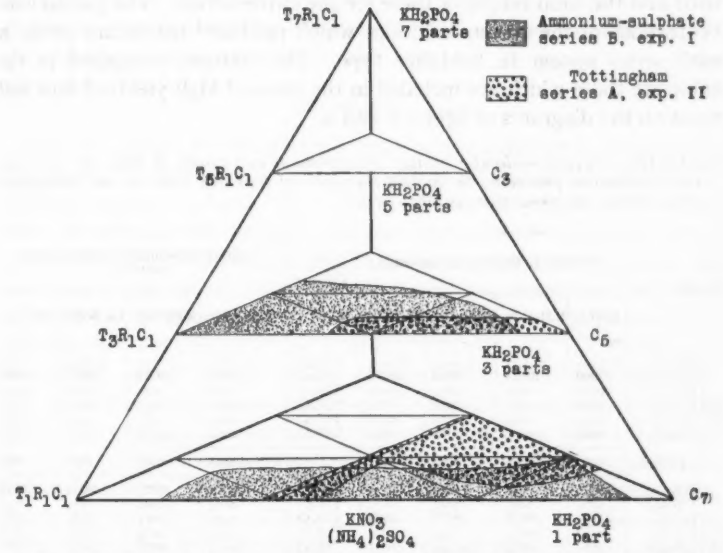


FIG. 9.—Distribution of relative dry-weight values of roots for the best six cultures in each series.

and $T_1R_1C_3$ in the Tottingham series and the ammonium-sulphate series, respectively. In each of these series, however, the maximum yield of tops and that of roots was produced by different cultures.

A comparison of the diagram of figure 8 with that of figure 9 shows a pronounced similarity and very close agreement between the areas representing high yields of tops and those representing high root yields for the ammonium-sulphate series. Five of the six cultures which are included in the areas of high top yields (fig. 8) also appear in the areas of high root yields (fig. 9). In the Tottingham series, however, no such marked similarity between the areas of high top yields and those of high root yields is apparent. In this series only two cultures appear in the areas of both high top yields and high root yields, although the detached

culture $T_3R_1C_5$, marking the lower limit in the range of high top yields, appears also in an area representing high root yields.

From the distribution of the areas representing high yields of tops and roots on the diagrams of figures 8 and 9, it is at once apparent that good growth may be correlated with relatively wide ranges in the proportions of most of the salts comprised in the culture solutions of the two series here considered, but for ammonium-sulphate a narrow range only is indicated. This is brought out best by the data in Table IX, which gives the volume-molecular partial concentrations of the salts and the ranges of these for the culture solutions in each series which produced the best six yields of tops and of roots. At the bottom of this table are given the maximum and minimum partial concentrations of each salt used and the total ranges of these for the entire series. The partial concentrations of the culture solutions which produced maximum yields in each series appear in bold-face type. The cultures comprised in the table are those which are included in the areas of high yields of tops and roots on the diagrams of figures 8 and 9.

TABLE IX.—*Volume-molecular partial concentrations and ranges of these for the salts in the solutions producing the highest six yields of tops and roots in the Tottingham series and in the ammonium-sulphate series*

	Culture No.	Tottingham Series (experiment II).				Culture No.	Ammonium-sulphate series (experiment I).			
		KNO_3.	KH_2PO_4.	$Ca(NO_3)_2$.	$MgSO_4$.		$(NH_4)_2SO_4$.	KH_2PO_4.	$Ca(NO_3)_2$.	$MgSO_4$.
Tops	$T_1R_1C_3$...	0.0020	0.0021	0.0044	0.0119	$T_1R_1C_3$	0.0014	0.0021	0.0044	0.0119
	$T_1R_3C_3$...	**.0060**	**.0021**	**.0044**	**.0071**	$T_1R_3C_5$	**.0014**	**.0021**	**.0073**	**.0071**
	$T_3R_1C_5$...	.0020	.0063	.0073	.0024	$T_1R_3C_3$	.0042	.0021	.0073	.0024
	$T_3R_3C_1$...	.0060	.0063	.0015	.0071	$T_3R_1C_3$	.0014	.0063	.0044	.0071
	$T_3R_3C_1$...	.0100	.0063	.0015	.0024	$T_3R_3C_1$	.0042	.0063	.0015	.0071
	$T_3R_3C_1$...	.0060	.0106	.0015	.0024	$T_3R_1C_1$	.0014	.0106	.0015	.0071
	Range..	.0080	.0085	.0058	.0095		.0028	.0085	.0058	.0095
Roots	$T_1R_1C_3$...	.0020	.0021	.0044	.0119	$T_1R_1C_3$	**.0014**	**.0021**	**.0044**	**.0119**
	$T_1R_3C_3$...	.0060	.0021	.0044	.0071	$T_1R_1C_5$	.0014	.0021	.0073	.0071
	$T_1R_3C_5$...	**.0060**	**.0021**	**.0073**	**.0024**	$T_1R_3C_5$	.0042	.0021	.0073	.0024
	$T_1R_3C_1$...	.0120	.0021	.0044	.0024	$T_3R_1C_1$	.0014	.0063	.0015	.0119
	$T_3R_1C_3$...	.0020	.0063	.0044	.0071	$T_3R_1C_3$	.0014	.0063	.0044	.0071
	$T_3R_1C_5$...	.0020	.0063	.0073	.0024	$T_3R_3C_1$	.0042	.0063	.0015	.0071
	Range..	.0080	.0042	.0029	.0095		.0028	.0042	.0058	.0095
Entire series:	Maximum.	.0140	.0148	.0102	.0166		.0098	.0148	.0102	.0166
	Minimum.	.0020	.0021	.0015	.0024		.0014	.0021	.0015	.0024
	Range.....	.0120	.0127	.0087	.0142		.0084	.0127	.0087	.0142

Inspection of the data in Table IX shows that the salt proportions characterizing the cultures which gave the highest six yields of tops and roots in the two series are limited to certain ranges in these proportions which are always less extensive than the total ranges for the entire series. The range in the proportions of each salt which produced high yields of tops in the Tottingham series is relatively wide, in each instance being approximately two-thirds of the total range of these proportions

for the entire series. In the ammonium-sulphate series the ranges in the proportions of monopotassium phosphate, calcium nitrate, and magnesium sulphate are in absolute agreement with the corresponding ranges in the proportions of these salts in the Tottingham series, while the range in the proportions of ammonium sulphate for high yields of tops is relatively low, being only one-third of the total range in the proportions of this salt for the entire series. This range includes the lowest proportion of this salt used in the series.

The cultures which produced high root yields in the Tottingham series are characterized by relatively wide ranges in the proportions of potassium nitrate and magnesium sulphate and relatively narrow ones in the proportions of monopotassium phosphate and calcium nitrate, while high yields of roots in the ammonium-sulphate series are associated with a narrow range in the proportions of ammonium sulphate and monopotassium phosphate and a wide range in the proportions of the other two salts.

SUMMARY

The experiments described in this paper were conducted for the purpose of studying, in a comparative way, the effects of ammonium sulphate in nutrient solutions upon the growth of young wheat plants and to determine the influence of this salt upon the ability of the plants to utilize iron from a soluble ferrous salt and an insoluble ferric salt. A study was also made of the change in reaction of the nutrient solutions induced by contact with the plant roots. Two series of culture solutions were used. The first of these comprised 20 solutions selected from the Tottingham series of 84, and the second series consisted of the same solutions modified by substituting ammonium sulphate for the potassium nitrate in equivalent osmotic concentrations. All the solutions had a total osmotic concentration value of approximately 1 atmosphere. The soluble and the insoluble iron in the form of ferrous sulphate and ferric phosphate, respectively, was added to the solutions in quantities of 0.83 mgm. of iron per liter of solution. The culture solutions were renewed at regular intervals of 3½ days throughout a total growth period of 35 days.

The main results of the experiments may be summarized as follows:

(1) The plants grown in the Tottingham solutions invariably produced a marked decrease in the hydrogen-ion concentrations of the solutions.

(2) The plants grown in the solutions containing ammonium sulphate invariably increased the hydrogen-ion concentration of these solutions during the early stages of growth. During the first five weeks of growth the hydrogen-ion concentrations were maintained at a much higher level in these solutions than in the unmodified Tottingham solutions, although the initial P_H values of corresponding solutions of the two types were practically the same.

(3) The direction of the change in reaction of the culture solutions produced by the growing wheat plants was determined by the nature of the salt constituents comprised in the solutions.

(4) Ferric phosphate, in the quantities used, was not sufficiently available in the Tottingham solutions to supply the needs of the plants for iron. The low yields produced by these solutions were correlated with a high degree of chlorosis and with high P_H values. On the other hand, this form of iron appears to be readily available to the plants in the solutions containing ammonium sulphate. The high yields produced by these solutions were associated with relatively low P_H values, and the plants were entirely free from chlorosis.

(5) Ferrous sulphate, in the quantities used, was sufficiently available in the Tottingham solutions to satisfy the needs of the plants for iron. These solutions with the soluble form of iron produced excellent growth and high yields, and no chlorotic or toxic effects were apparent. The solutions containing ammonium sulphate with this form of iron in quantities of more than 0.01 mgm. of iron per liter of nutrient solution were very toxic to the plants, the degree of toxicity increasing with increase in the amounts of iron added to the solutions.

(6) The nature of the nutrient solution with respect to the salt constituents and hydrogen-ion concentration appears to determine the availability and the efficiency of a given iron salt for plant growth.

(7) The highest six yields of wheat tops from the Tottingham series are correlated with relatively wide ranges in the proportions of all four salts employed. The maximum yield of tops from these series was produced by a solution having the following salt proportions: Potassium nitrate 0.0060 M.; potassium phosphate 0.0021 M.; calcium nitrate 0.0044 M.; and magnesium sulphate 0.0071 M., with ferrous sulphate (0.83 mgm. of iron per liter) as the source of iron for the plants.

(8) The highest six yields of tops from the series of solutions containing ammonium sulphate are associated with relatively wide ranges in the proportions of potassium phosphate, calcium nitrate, and magnesium sulphate, but with a narrow range in the proportions of ammonium sulphate. The maximum yield of tops from these series was produced by a solution having the following salt proportions: Ammonium sulphate 0.0014 M.; potassium phosphate 0.0021 M.; calcium nitrate, 0.0073 M.; and magnesium sulphate 0.0071 M., with ferric phosphate (0.83 mgm. of iron per liter) as the source of iron for the plants.

(9) In the series of solutions containing ammonium sulphate, high yields of tops were generally associated with high yields of roots; but no such correlation between tops and roots existed in the Tottingham series.

LITERATURE CITED

(1) CLARK, William Mansfield, and LUBS, Herbert A.
1917. THE COLORIMETRIC DETERMINATION OF HYDROGEN ION CONCENTRATION AND ITS APPLICATIONS IN BACTERIOLOGY. *In* Jour. Bact., v. 2, no. 1, p. 1–34; no. 2, p. 109–136; no. 3, p. 191–236, 9 fig. (1 fold.) References, p. 233–236.

(2) DUGGAR, B. M.
1920. HYDROGEN ION CONCENTRATION AND THE COMPOSITION OF NUTRIENT SOLUTIONS IN RELATION TO THE GROWTH OF SEED PLANTS. *In* Ann. Mo. Bot. Gard., v. 7, no. 1, p. 1–49. Bibliography, p. 47–49.

(3) ESPINO, Rafael B.
1920. SOME ASPECTS OF THE SALT REQUIREMENTS OF YOUNG RICE PLANTS. *In* Philippine Jour. Sci., v. 16, no. 5, p. 455–525, 9 fig., 1 pl.

(4) FREE, E. E.
1915. A RELATIVE SCORE METHOD OF RECORDING COMPARISONS OF PLANT CONDITIONS AND OTHER UNMEASURED CHARACTERS. *In* Plant World, v. 18, no. 8, p. 249–256, 3 fig.

(5) GILE, P. L., and CARRERO, J. O.
1916. ASSIMILATION OF IRON BY RICE FROM CERTAIN NUTRIENT SOLUTIONS. *In* Jour. Agr. Research, v. 7, no. 12, p. 503–528. Literature cited, p. 528.

(6) ——— ———
1920. CAUSE OF LIME-INDUCED CHLOROSIS AND AVAILABILITY OF IRON IN THE SOIL. *In* Jour. Agr. Research, v. 20, no. 1, p. 33–62, pl. 5–6. Literature cited, p. 59–61.

(7) HARTWELL, Burt L., and PEMBER, F. R.
1908. THE RELATIVE TOXICITY OF FERROUS SULPHATE TO BARLEY AND RYE SEEDLINGS. *In* R. I. Agr. Exp. Sta. 21st Ann. Rpt., 1907/08, p. 286–294.

(8) HOAGLAND, D. R.
1917. THE EFFECT OF HYDROGEN AND HYDROXYL ION CONCENTRATION ON THE GROWTH OF BARLEY SEEDLINGS. *In* Soil Sci., v. 3, no. 6, p. 547–560. Literature, p. 559–560.

(9) ———
1918. THE RELATION OF THE PLANT TO THE REACTION OF THE NUTRIENT SOLUTION. *In* Science, n. s. v. 48, no. 1243, p. 422–425.

(10) ———
1919. RELATION OF THE CONCENTRATION AND REACTION OF THE NUTRIENT MEDIUM TO THE GROWTH AND ABSORPTION OF THE PLANT. *In* Jour. Agr. Research, v. 18, no. 2, p. 73–117, 4 fig. Literature cited, p. 114–117.

(11) HUTCHINSON, H. B., and MILLER, N. H. J.
1909. DIRECT ASSIMILATION OF AMMONIUM SALTS BY PLANTS. *In* Jour. Agr. Sci., v. 3, pt. 2, p. 179–194, 2 fig., pl. 14. References, p. 193–194.

(12) JONES, Linus H., and SHIVE, John W.
1921. THE INFLUENCE OF IRON IN THE FORMS OF FERRIC PHOSPHATE AND FERROUS SULPHATE UPON THE GROWTH OF WHEAT IN A NUTRIENT SOLUTION. *In* Soil Sci., v. 11, no. 2. p. 93–99, 1 fig., 1 pl. (in text).

(13) PANTANELLI, E.
1915. ÜBER IONENAUFNAHME. *In* Jahrb. Wiss. Bot. [Pringsheim], Bd. 56, p. 689–733. Literatur, p. 730–733.

(14) RUPRECHT, R. W.
1915. TOXIC EFFECT OF IRON AND ALUMINUM SALTS ON CLOVER SEEDLINGS. *In* Mass. Agr. Exp. Sta. Bul., 161, p. 125–129, 1 pl.

(15) SHIVE, John W.
1915. A STUDY OF PHYSIOLOGICAL BALANCE IN NUTRIENT MEDIA. *In* Physiol. Researches, v. 1, no. 7, p. 327–397, 15 fig. Literature cited, p. 396–397.
(16) THATCHER, Roscoe W.
1921. THE CHEMISTRY OF PLANT LIFE. xvi, 268 p. New York, London. References at end of most of the chapters.
(17) TOOLE, E. H., and TOTTINGHAM, W. E.
1918. THE INFLUENCE OF CERTAIN ADDED SOLIDS UPON THE COMPOSITION AND EFFICIENCY OF KNOP'S NUTRIENT SOLUTION. *In* Amer. Jour. Bot., v. 5, no. 9, p. 452–461. Literature cited, p. 460–461.
(18) TOTTINGHAM, William E.
1914. A QUANTITATIVE CHEMICAL AND PHYSIOLOGICAL STUDY OF NUTRIENT SOLUTIONS FOR PLANT CULTURES. *In* Physiol. Researches, v. 1, no. 4, p. 133–245, 15 fig. Literature cited, p. 242–245.
(19) TRELEASE, Sam F.
1920. THE GROWTH OF RICE AS RELATED TO PROPORTIONS OF FERTILIZER SALTS ADDED TO SOIL CULTURES. *In* Philippine Jour. Sci., v. 16, no. 6, p. 603–629, 5 fig. References, p. 627.
(20) ——— and PAULINO, Pedro.
1920. THE EFFECT ON THE GROWTH OF RICE OF THE ADDITION OF AMMONIUM AND NITRATE SALTS TO SOIL CULTURES. *In* Philippine Agr., v. 8, no. 8/9, p. 293–313. Bibliography, p. 313.
(21) WOLKOFF, M. I.
1918. EFFECT OF AMMONIUM SULFATE IN NUTRIENT SOLUTIONS ON THE GROWTH OF SOYBEANS IN SAND CULTURES. *In* Soil Sci., v. 5, no. 2, p. 123–150, 7 fig. References, p. 149–150.

XI, No. 10

Physiol.
396–397.

London,

OSITION
. Jour.

TRIENT
v. 1,

TILIZER
v. 16,

IONIUM
, v. 8,

N THE
no. 2,

PLATE 136

A.—Ferric phosphate as the source of iron for plants, 0.75 mgm. of iron per liter of nutrient solution.

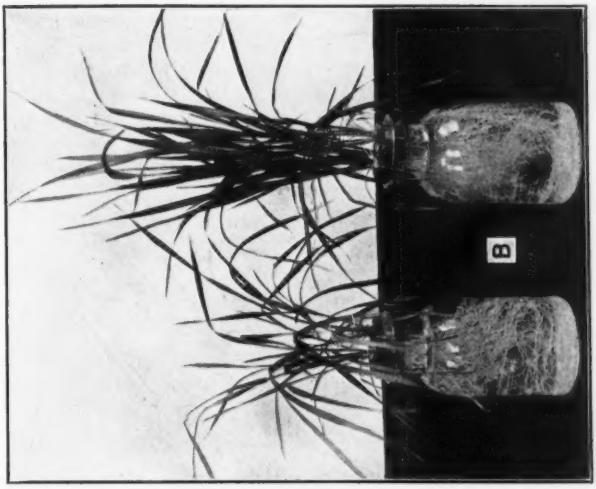
The plants in the culture on the left were grown in the Tottingham solution $T_3R_1C_5$ modified by substituting ammonium sulphate for the potassium nitrate in equivalent osmotic concentrations. The plants are green and healthy.

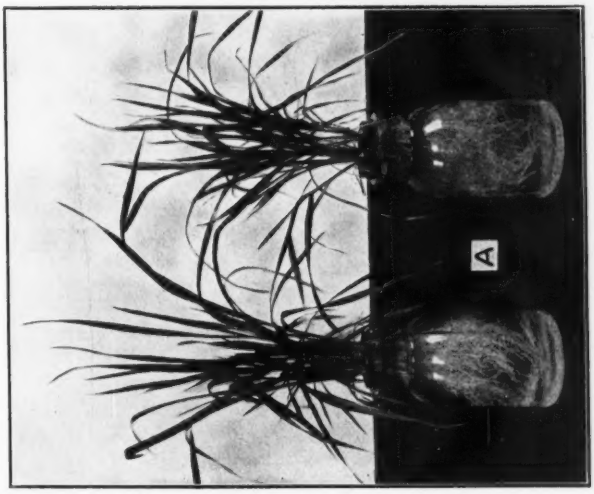
The plants in the culture on the right were grown in the Tottingham solution $T_3R_1C_5$ unmodified. The plants are chlorotic and much smaller than those grown in the solution containing ammonium sulphate.

B.—Ferrous sulphate as the source of iron for plants, 0.75 mgm. of iron per liter of nutrient solution.

The plants in the culture on the left were grown in the Tottingham solution $T_3R_1C_5$ modified by substituting ammonium sulphate for the potassium nitrate in equivalent osmotic concentrations. The plants are smal! and unhealthy.

The plants in the culture on the right were grown in the Tottingham solution $T_3R_1C_5$ unmodified. The plants are large, green, and healthy.


B


A

DISPERSION OF FLIES BY FLIGHT

By F. C. BISHOPP, *Entomologist*, and E. W. LAAKE, *Entomological Assistant, Investigations of Insects Affecting the Health of Man and Animals, Bureau of Entomology, United States Department of Agriculture*

A definite knowledge of the means of dissemination of various species of economic insects is of much importance in control or eradicative undertakings. Undoubtedly the spread of injurious forms by artificial means is in general of most pronounced importance. With free-flying species, however, natural dispersion deserves careful consideration. This is particularly true of the various species of flies which directly affect man and animals. The accumulation of data on the possibilities of flight of various injurious species of flies should aid economic work in several ways.

1. It should help in the study of the spread of fly-borne diseases, either in large districts or locally.

2. It should make possible the proper location of dumps, incinerators, hog-feeding stations, and other favorable breeding grounds so that the menace to towns, cities, military camps, etc., will be reduced to a minimum.

3. In control work in restricted districts or about individual plants it should help in determining the extent to which fly-breeding grounds in the neighborhood affect these undertakings.

4. In the prosecution of large-scale control work against certain species on farms or ranges, it should show how widespread must be the effort if marked results are to be accomplished.

5. It should make it possible to determine whether control campaigns are accumulative from year to year in their effect, or whether, owing to extensive migration, the results are effective only during one year or one period.

Realizing the desirability of collecting data along this line, a number of investigators in various parts of the world have carried out dispersion tests. These were mainly with the house fly (*Musca domestica* L.), although limited numbers of a few other species were observed in the experiments. Most of these tests were conducted under more or less distinctly urban conditions. The observations and main conclusions of these various experimenters have been so well summarized by Parker [1] that they will not be repeated here. Parker also discusses rather fully in the same article the methods of marking the flies in the tests.

The maximum range of dispersion recorded up to the work of Parker was 1,700 yards. This distance was noted in a test in England carried

[1] PARKER, R. R. DISPERSION OF MUSCA DOMESTICA LINNAEUS UNDER CITY CONDITIONS IN MONTANA. *In* Jour. Econ. Ent., vol. 9, no. 3, p. 325–354, pl. 24–26. 1916.

out under urban conditions by Copeman, Howlett, and Merriman in 1911.[1]

Parker's experiments in Montana were also carried out largely under city conditions. The greatest range of dispersion shown in his tests was 3,500 yards, or nearly 2 miles, with the house fly. A record of 500 yards was made for *Muscina stabulans* Fall., and 160 yards for the black blowfly (*Phormia regina* Meig.). These last species, however, were released in comparatively insignificant numbers.

In addition to exact evidence obtained by recapturing marked and released flies, other evidence of dispersion is at hand. Hodge[2] has observed house flies, stable flies (*Stomoxys calcitrans* L.), and "blue bottles" in considerable numbers at cribs 5 and 6 miles out in Lake Erie north of Cleveland, Ohio. He concludes that the flies appear to be blown out at least 6 miles off shore. In 1917, Ball[3] presented evidence which indicates that the house fly may go distances of 45 or 95 miles. These observations were made at the Rebecca Light Shoal off the coast of Florida, and the conclusion was reached that the flies came down wind from Cuba (95 miles distant) and at times from the Marquesas Keys (24 miles distant) or possibly from Key West, Fla. (46 miles distant). While the evidence of flight over these long distances is convincing it is hardly incontrovertible. The flight in these instances was over water, at least for the most part; hence the conditions were very different from those existing under usual urban or rural conditions.

With a view to securing some definite facts as to the tendencies and possibilities of dispersion under rural conditions, a series of tests with injurious species was carried out in northern Texas during the summers of 1916 and 1918.

METHOD OF CATCHING, MARKING, AND LIBERATING FLIES

Practically the same method of handling the flies was used throughout the several tests conducted. The supply was secured by baiting large conical flytraps with "gut slime," a packing house by-product. These traps were left in operation from a few hours up to 24 hours; then a screen cylinder was placed on top of the flytrap. The cover of the trap was removed, and the flies were agitated so that they would pass upward into the cylinder, which was first supplied with a number of green branches from trees. Few of the weaker, disabled flies would pass upward into the cylinders. When several thousand had passed into the cylinder a cheesecloth was placed over the lower end of it. All the flies were

[1] COPEMAN, S. Monckton, HOWLETT, F. M., and MERRIMAN, Gordon. AN EXPERIMENTAL INVESTIGATION ON THE RANGE OF FLIGHT OF FLIES. *In* Rpts. Local Govt. Bd. [Gt. Brit.] Pub. Health and Med. Subjs., n. s. no. 53, p. 1–9. 1911.

[2] HODGE, C. F. THE DISTANCE HOUSE FLIES, BLUE BOTTLES AND STABLE FLIES MAY TRAVEL OVER WATER. *In* Science, n. s., v. 38, no. 980, p. 512–513. 1913.

[3] BALL, S. C. MIGRATION OF INSECTS TO REBECCA SHOAL LIGHT-STATION AND THE TORTUGAS ISLANDS, WITH SPECIAL REFERENCE TO MOSQUITOES AND FLIES. *In* Papers Dept. Mar. Biol. Carnegie Inst. Wash., v. 12 (Publ. 252), p. 193–212. 1918. Bibliography, p. 212.

captured in Dallas or Fort Worth at the packing houses and were then transported to the point of liberation. The time from placing the flies in the cylinders until they were liberated ranged from 1 to 2 hours.

When the point of liberation was reached the cylinders were slipped into a canvas bag one at a time and from 1 to 2 ounces of finely powdered red chalk or paint pigment were introduced into the cage, which was inverted several times. The top was then removed, and the flies were allowed to escape. All flies were then shaken from the cages.

The number and percentage of the different species of flies released were estimated by looking into the screened cylinders. While it is realized that this estimate is very rough, the writers' experience in handling great numbers of flies in traps has added to the reliability of the estimates. In one instance the flies in one trap were killed, and the proportion of species and sex was determined by actual count.

When the flies emerged from the cage they were all distinctly covered with the chalk, the majority of them being brilliant red. In one instance yellow chalk was used and in another the flies were sprayed with rosalic acid, but the yellow color was not so readily distinguished in the mass of flies caught in the recovery traps, and not a single fly sprayed with rosalic acid was identified when an alkaline solution was applied to the specimens taken in the recovery traps. In a few of the liberations a red paint pigment was used, and this also seemed fairly satisfactory.

In every case there was a considerable mortality among the flies placed in the cages, but it is believed that this was caused more by the heat while the specimens were being transported to the place of liberation than by the application of the marking agent. That there may have been some deleterious effect from the application of the chalk dust can not be denied, but the fact that some marked specimens were recovered 17 days after application indicates that the method of marking was not highly injurious.

ACTION OF FLIES WHEN LIBERATED

All liberations were made on the ground in open fields. In every instance a considerable number of the flies were observed to take to the air immediately, some passing upward to a considerable height. They seemed to fly freely in all directions, but there seemed to be more going with the wind or at right angles to it than in other directions. A large number settled on the grass near by, and many of these were observed to be preening themselves in a contented way. In experiments where trees were near at hand large numbers were observed to settle on the leaves. Many flies exhibited great thirst, as was shown by efforts to procure moisture from the leaves and perspiration on our bodies. They persistently stuck to our clothing, hands, and faces, and, although the conveyance used was always left some distance from the point of liberation, a few flies were in every instance observed to be

about the vehicle when the start away from the point was made. The flies were at first driven off, and after a short distance had been traveled all of those about the vehicle were killed. A second and sometimes a third examination were made to make sure that no colored specimens were following.

RECOVERY AND IDENTIFICATION OF FLIES

In order to determine the distance of dissemination, conical traps 18 inches in diameter baited with "gut slime" were set at measured distances in different directions from the point of liberation. The flies collected in these recovery traps were killed at daily intervals as nearly as possible, and the mass of flies was carefully gone over for colored specimens. In most instances the colored flies could be identified with the unaided eye, but when any doubtful specimens were found they were examined with a microscope and the presence of particles of chalk could then be determined accurately. During the first few days after liberation most of the flies were strongly colored, but later the specimens retained the color mainly on the halteres.

The sex and species of the marked flies recovered were determined, and in many cases the percentage of the different species of unmarked flies in the recovery traps was estimated by examination of a certain number of flies, and in the same way the sexes were estimated in a number of instances. The weight of the catch in each recovery trap was also determined.

Owing to the different conditions under which the several dissemination tests were made it seems best to discuss them separately.

INITIAL FLIGHT TEST NEAR FORT WORTH, TEX.

On May 31, 1916, at 1.30 p. m., 7,000 or 8,000 flies were marked with red chalk and liberated in an open field about ½ mile east of the two large packing houses which are located approximately 2 miles north of Fort Worth. One of the prime objects of this test was to determine the freedom with which the flies would go toward packing houses from surrounding areas where more or less attractive feeding and breeding conditions for flies of all kinds occurred. Eighteen recovery traps were used in this test. They were set at distances and directions as follows from the point of liberation (fig. 1): Trap No. 1, 930 feet 15° north of west on dock of a silo construction company; trap No. 2, 851 feet 15° south of west on a platform of a plant where dead animals were rendered, hence very attractive for flies; trap No. 3, 1,037 feet southwest near a small slaughterhouse with surroundings attractive for flies; trap No. 4, 1,323 feet 12° south of west in garden behind store; traps No. 5, 6, 7, and 8, about 2,123 feet 25° south of west located around a rendering plant where conditions were attractive to flies; traps No. 9, 10, 11, 12, and 13, about 2,588 feet 5° south of west and within the inclosure of one of the large

packing houses; traps No. 14 to 18, inclusive, 3,076 feet 5° north of west within the inclosure of another large packing house. To make traps No. 2 and 3 still more attractive to blowflies a large amount of meat waste was hauled out into the field between them and partially plowed under.

A creek fringed with trees flows nearly north between the point of liberation and the packing houses, about 1,100 feet from the point of liberation; thus the flies, in order to reach traps No. 4 to 18, inclusive, would have to cross this stream. The closest road with any considerable amount of travel is about 1,400 feet from the point of liberation, passing near traps No. 5 to 13 and turning north at right angles near trap No. 4.

Almost immediately after release two colored screw-worm flies (*Chrysomya macellaria* Fab.) were observed at trap No. 1, and several were seen about the rendering plant at trap No. 2. About three hours after liberation a marked *C. macellaria* was observed through the screen in trap No. 5.

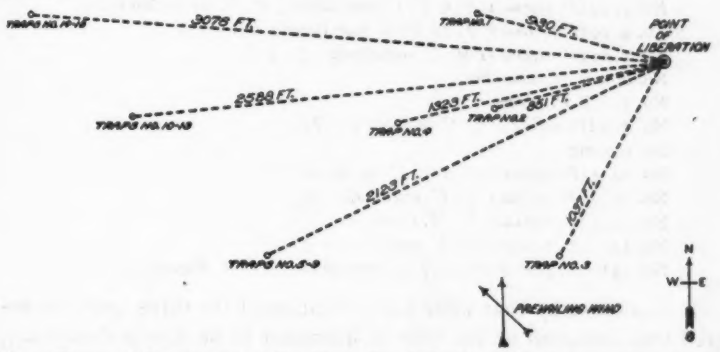


FIG. 1.—Diagram of dispersion test at Fort Worth, Tex., indicating relation of recovery traps to point of release.

June 1, 11.45 a. m., flies in traps No. 10 to 13, inclusive, were killed and examined and the following marked flies found: Trap No. 10, 1 ♀ *Phormia regina* (the black blowfly), 1 ♀ *Chrysomya macellaria;* No. 11, 1 ♀ *P. regina;* No. 12, 1 ♀ *P. regina;* No. 13, 2 ♀ *P. regina*, 1 ♀ *C. macellaria*. June 1, between 2 and 3.45 p. m., the following colored flies were found in traps No. 1 to 9, inclusive: No. 1, 2 ♂ and 16 ♀ *P. regina*, 1 ♀ *C. macellaria*, 5 ♀ *Musca domestica* (house fly); No. 2, 11 ♀ *P. regina*, 1 ♀ *M. domestica;* No. 3, 1 ♂ and 5 ♀ *P. regina*, 3 ♀ *C. macellaria*, 5 ♀ *M. domestica;* No. 4, 2 ♂ and 9 ♀ *P. regina*, 2 ♀ *C. macellaria*, 2 ♀ *M. domestica;* No. 5, 6 ♀ *P. regina*, 3 (2 ♀, 1?) *C. macellaria*. No. 6, 7, 8, 9, none. June 2, 9.45 to 11.20 a. m., No. 14 to 18 contained the following colored flies: 4 ♀ *P. regina*, 2 ♀ *C. macellaria*, 1 ♀ *M. domestica*. June 2, 12.30 to 2 p. m., flies in No. 5 to 13, inclusive, were killed and examined, and the following marked specimens were found: No. 5, 4 ♀ *P. regina*, 1 ♀ *C. macellaria;* No. 6, 1 ♀ *M. domestica;* No. 7, none; No. 8, 2 ♀ *P. regina;* No. 9, none;

No. 10, 1 ♂, 1 ♀ *P. regina;* No. 11, none; No. 12, 1 ♀ *M. domestica;* No. 13, none. June 2, 2.30 p. m., No. 1, 1 ♀ *P. regina,* 1 ♀ *C. macellaria;* No. 2, 5 ♀ *P. regina,* 1 ♀ *C. macellaria* (sex ?), 2 ♀ *M. domestica;* No. 3, 2 ♀ *P. regina,* 1 ♀ *M. domestica;* No. 4, 2 ♀ *P. regina;* No. 5, none. June 3, 9.30 to 11.20 a. m., No. 5, none; No. 6, 1 ♀ *M. domestica;* No. 7, 2 ♀ *P. regina;* No. 8, 9, and 10, none; No. 11, 1 ♀ *C. macellaria;* No. 12, 1 ♀ *P. regina;* No. 13, none. June 3, 1.30 p. m., No. 1, none; No. 2, 1 ♀ *C. macellaria;* No. 3, 1 ♀ *P. regina;* No. 4, 1 ♀ *C. macellaria.* June 5, 10 a. m., No. 1 and 2, none; No. 3, 1 ♀ *P. regina;* No. 4, 3 ♂ *P. regina;* No. 5, 1 ♀ *P. regina;* No. 6 and 7, none; No. 8, 1 ♀ *C. macellaria;* No. 9 to 14, inclusive, none.

The grand total of all the colored flies caught in the traps was 126, distributed among the traps as follows:

No. 1, 26 (*P. regina* 2 ♂, 17 ♀; *C. macellaria* 2 ♀; *M. domestica* 5 ♀).
No. 2, 21 (*P. regina* 16 ♀; *C. macellaria* 1 ♀, 1 ?; *M. domestica* 3 ♀).
No. 3, 19 (*P. regina* 1 ♂, 9 ♀; *C. macellaria* 3 ♀; *M. domestica* 6 ♀).
No. 4, 19 (*P. regina* 2 ♂, 14 ♀; *C. macellaria* 3 ♀).
No. 5, 15 (*P. regina* 11 ♀; *C. macellaria* 3 ♀, 1 ?).
No. 6, 2 ♀ *M. domestica.*
No. 7, 2 ♀ *P. regina.*
No. 8, 3 (*P. regina* 2 ♀; *C. macellaria* 1 ♀).
No. 9, none.
No. 10, 4 (*P. regina* 1 ♂, 2 ♀; *C. macellaria* 1 ♀).
No. 11, 2 (*P. regina* 1 ♂; *C. macellaria* 1 ♀).
No. 12, 3 (*P. regina* 2 ♀; *M. domestica* 1 ♀).
No. 13, 3 (*P. regina* 2 ♀; *C. macellaria* 1 ♀).
No. 14 to 18, 7 (*P. regina* 4 ♀; *C. macellaria* 2 ♀; *M. domestica* 1 ♀).

It is noteworthy that while the percentage of the three species recovered was estimated at the time of liberation to be *Musca domestica* 7, *Chrysomya macellaria* 70, *Phormia regina* 22, the percentage of these species as recovered was approximately *M. domestica* 14, *C. macellaria* 16, *P. regina* 70. At first sight this might lead one to think that *P. regina* was more prone to migration than *C. macellaria;* however, when we group the first four traps, which were from 930 to 2,588 feet from the point of liberation, and the last 14, which were from 2,123 to 3,076 feet, exactly 50 per cent of the total number of marked *C. macellaria* recovered were in the more distant traps while the percentage of marked *P. regina* was but slightly over 30.5. Comparing the house flies in the same way, about 22 per cent were taken in the more distant group of traps. Attention might also be called to the fact that of the 126 marked flies recovered only 6, or 4.75 per cent, were males. The percentage of the sexes of the flies liberated was not determined, but in the usual catch the number of males is much greater than this. Five of these males were taken in the four nearest traps, which might further suggest that the tendency to distant dissemination is more marked in the females.

It is notable that over 64 per cent of the total number of marked flies recovered were found to be captured when the traps were emptied about 24 hours after the liberation had been made, and several of the traps were not examined within this 24-hour period.

This test also shows that distances up to 1,000 feet are covered in a few minutes by flies, that the screw-worm fly may travel nearly half a mile in 3 hours, and that the house fly may go over 3,000 feet in less than 48 hours.

Since the recovery traps were not placed in all directions from the point of liberation, it is very difficult to draw conclusions in this test in regard to the effect of wind on dissemination. During this recovery period the wind during the daytime varied in velocity from 5 to 30 miles, and the prevailing directions were south and southeast. (Table I.) The wind with a velocity of from 13 to 24 miles blew from the southeast and south during the 48 hours following release, and since the majority of the marked flies recovered were taken within that period it is seen that those captured traveled practically at right angles to this wind.

A second liberation of about 10,000 flies marked with yellow chalk was made at 4.30 p. m., June 1. The proportion of the different species was estimated to be as follows: *Chrysomya macellaria* 60 per cent, *Phormia regina* 28 per cent, *Musca domestica* 10 per cent, other species, including Lucilia, Ophyra, Sarcophaga, and *Muscina stabulans*, 2 per cent. Difficulty was encountered in identifying the flies from this liberation. None were observed except on June 2, when the following recoveries were positively made: No. 14, 10.15 a. m., 1 ♀ *P. regina;* No. 10, 12.30 p. m., 1 ♀ *P. regina;* No. 11, 2 ♀ *P. regina*, 1 ♀ *C. macellaria;* No. 13, 1 ♂ *P. regina*, 1 ♀ *C. macellaria;* No. 1, 1.30 p. m., 2 ♂ and 1 ♀ *P. regina;* No. 2, 1 ♀ *C. macellaria.* This shows that *P. regina* traveled over 3,000 feet in less than 18 hours after release and that night hours were included in this period.

TABLE I.—*Climatological data relating to initial dispersion test at Fort Worth, Tex.*

Date.	Temperature.			Wind.		Rain.	Humidity.			Actual sun.
	Max.	Min.	Mean.	Direction during successive hours from 6 a. m. to 8 p. m.	Velocity during day.		Max.	Min.	Mean.	
1916.	° F.	° F.	° F.		Miles.	Inch.	Per cent.	Per cent.	Per cent.	Per cent.
May 31....	94	70	82	14 SE.....	8 to 24	0	76	39	56	92
June 1....	95	72	84	14 S.....	15 to 24	0	75	34	54	79
2....	96	75	86	2 S., 4 SW., 2 NW., 3 W., 3 NW.	5 to 21	0	76	43	60	87
3....	94	72	83	2 NE., 9 SE., 1 S., 2 SE.....	5 to 13	0	70	49	60	80
4....	87	75	81	14 SE.....	8 to 26	0	77	50	64	52
5....	86	70	78	2 S., 5 SW., 5 W., 2 NW.....	19 to 30	0	68	39	54	73

FIRST DISPERSION TEST NEAR DALLAS, TEX.

On June 29, 1916, about 18,000 flies, consisting of approximately 70 per cent *Musca domestica*, 25 per cent *Chrysomya macellaria*, 1 per cent *Phormia regina*, and 4 per cent made up of *Lucilia sericata* Meig., *Muscina stabulans*, *Sarcophaga* spp., and *Ophyra* spp. were liberated. These flies were marked just before liberation with red chalk in the manner previously described. The point chosen for liberation was in the country about 4 miles east of the edge of the city of Dallas and within a few hundred feet of a large orphanage. Sixteen recovery traps baited with "gut slime" were set along the roads leading approximately east and west and north and south from the point of liberation. Four traps were set in each direction, the outer ones being between 2 and 3 miles from the point of liberation. The distance of each trap from this point is shown in Table II. The country covered by this radius might be described as a rolling, black land, farmed area. In general, farmhouses were located from ½ to 1 mile apart and most of them maintained barns and pigpens. The country was practically open, there being only a few narrow strips of woods in the area covered. The orphanage, with a number of dwellings and two or three stores at the corner, all near the point of liberation, might be considered the most attractive point for house flies in the area.

The environment of the traps undoubtedly affects the character of the entire catch and also the tendency for flies in their dissemination to linger near them and perhaps ultimately be captured. For this reason the surroundings of each trap are briefly given.

West, No. 1, behind small grocery store, some refuse, no barns near; No. 2, under tree 50 yards from dairy barn and pen; No. 3, under tree near farmhouse, a few head of stock kept near; No. 4, by side of dairy barn, favorable conditions for house flies. North, No. 1, in old pigpen near farmhouse; No. 2, in weeds near road and trees, farmhouse 75 yards away; No. 3, in cornfield, about ⅓ mile from any farmhouse, no attractive places near; No. 4, near farmhouse and barns, about ¾ mile south of the village of Reinhardt. East, No. 1, under shed at gin, horses in lot and farm about 50 yards away; No. 2, in edge of cornfield, no buildings nearer than ⅓ mile; No. 3, in tall grass near small stream, nearest building about 1/5 mile; No. 4, in old barnyard, numerous livestock in adjacent lot. South, No. 1, in cornfield ⅓ mile from southernmost buildings of Orphans' Home, no others nearer; No. 2, under shed by grocery store, several houses and Texas & Pacific Railway near; No. 3, in weeds in garden, farmhouse 40 yards away and several others in vicinity; No. 4, under tree, farmhouse and barns 50 yards south.

The pike road running east and west from the point of liberation is heavily traveled by all kinds of vehicles, but especially automobiles. The road to the south has a moderate amount of travel and the road to

the north light travel. Freighting trucks and dairy wagons pass on the east and west road.

On June 30, at 11.00 a. m., a second liberation consisting of about 25,000 flies marked with red chalk was made at the same point as the first. The species in this lot were in about the same proportions as those in the first release, with possibly somewhat fewer house flies and a few more screw-worm flies and *Phormia regina*.

Immediately after these flies were liberated the flies in the recovery traps were killed and removed for examination, and these examinations were made at approximately daily intervals up to and including July 11, with the exception of July 2, 4, and 9. The bait pans were replenished each day that the flies were removed from the traps.

It is interesting to note that the proportion of the species among the marked flies recovered is very similar to the proportion of those species among the flies released. These figures are given for comparison: *Musca domestica*, 70 per cent released, 77 per cent recovered; *Chrysomya macellaria*, 25 per cent released, 21 per cent recovered; *Phormia regina*, 1 per cent released, 0.83 per cent recovered; *Lucilia* spp., *Ophyra* spp., *Sarcophaga* spp., *Synthesiomyia brasiliana* Brauer and Bergenstamm, 1 per cent released, 1.16 per cent recovered. It should be remembered that the percentages of the species released are only a rough estimate. The percentage of the different species in the total catch during the recovery period was not determined; hence it can not be compared with the marked flies released and captured.

DISTANCE OF DISPERSION

The essential data in these experiments as regards the number of flies recovered in traps set at different distances in different directions are presented in Table II. It will be noted that in general the closer rings of traps caught more flies than those at greater distances, although in the outside circle of traps the number of flies taken was greater than in the third ring. This may undoubtedly be explained, at least partially, on the basis of the more advantageous positions where some of the traps in the fourth circle were set. The influence of immediate environment on the recovery of marked flies will be discussed under some of the subsequent experiments.

TABLE II.—*First dispersion test at Dallas, Tex. Species and sex of marked flies recovered in rings of traps about point of liberation*

Trap No.	Distance from place of release.	*Musca domestica.*		*Chrysomya macellaria.*		*Phormia regina.*		*Lucilia sericata.*		*Lucilia caesar.*		*Ophyra leucostoma.*		*Synthesiomyia brasiliana.*		*Sarcophaga* spp.		Undetermined.		Total.		Grand total.
	Miles.	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
1 W	0.4	165	472	61	64	1	0	0	2	0	0	0	4	0	0	0	5	0	2	227	549	776
1 N	.5	124	1,082	53	122	1	2	0	6	0	0	1	4	0	2	0	0	0	0	179	1,218	1,397
1 E	.5	65	932	1	13	0	2	0	4	0	1	0	0	0	1	0	2	0	0	66	955	1,021
1 S	.5	3	146	24	40	0	4	0	1	0	0	0	2	0	0	1	1	0	0	28	194	222
Total		357	2,632	139	239	2	8	0	13	0	1	1	10	0	3	1	8	0	2	500	2,916	3,416
2 W	1.0	11	59	36	64	0	4	0	1	0	0	0	0	0	0	0	3	0	1	47	132	179
2 N	1.1	2	34	25	71	0	3	0	0	0	1	0	1	0	0	0	0	0	0	27	110	137
2 E	1.2	2	27	3	16	0	2	0	0	0	0	0	0	0	0	0	1	0	0	5	46	51
2 S	1.2	6	153	1	6	0	0	0	1	0	0	0	0	0	0	0	0	0	0	7	160	167
Total		21	273	65	157	0	9	0	2	0	1	0	1	0	0	0	4	0	1	86	448	534
3 W	1.6	7	55	21	57	1	8	0	0	0	1	0	0	0	0	0	1	0	0	29	122	151
3 N	1.8	0	31	6	56	0	2	0	0	0	0	0	0	0	0	0	0	0	0	6	89	95
3 E	2.0	0	7	19	38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	45	64
3 S	2.0	1	11	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	26	27
Total		8	104	46	166	1	10	0	0	0	1	0	0	0	0	0	1	0	0	55	282	337
4 W	2.1	14	106	17	44	1	0	0	0	0	0	0	0	0	0	0	0	0	1	32	151	183
4 N	2.3	3	49	10	45	2	5	0	0	0	0	0	1	0	0	0	1	0	0	15	101	116
4 E	3.5	1	25	6	15	0	0	0	0	1	0	0	0	0	0	0	0	0	0	8	40	48
4 S	3.1	2	9	2	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	4	14	18
Total		20	189	35	108	3	6	0	0	1	0	0	1	0	0	0	1	0	1	59	306	365
Grand total		406	3,198	285	670	6	33	0	15	1	3	1	12	0	3	1	14	0	4	700	3,952	4,652

DIRECTION OF DISPERSION

The data obtained in regard to the direction of flight were interesting. The total number of marked flies recovered in the different directions from the point of liberation was as follows: West, 1,289; north, 1,745; east, 1,184; south, 434. Of course the different species should really be discussed separately in treating of the direction or distance of dispersion; the figures show, however, that for some reason the liberated flies of all species failed to be recovered in the traps south of the point of liberation in numbers in proportion to those recovered in the other directions. Considering the house fly and screw-worm fly in regard to the numbers traveling in different directions, it will be seen in the case of the house fly that 889 (24.66 per cent) were recovered in the west traps, 1,325 (36.76 per cent) in the north, 1,067 (29.38 per cent) in the east, and 331 (9.18 per cent) in the south. In the case of the screw-worm fly 364 (38.11 per cent) were recovered in the west, 388 (40.62 per cent) in the north, 111 (11.62 per cent) in the east, and 92 (9.63 per cent) in the south traps. In an attempt to correlate these figures with the direction of the wind it appears that there is a tendency exhibited in this test for both of these species to go with the wind rather than against it, as will be seen by referring to climatological data in Table III. The prevailing wind during the first five days was from the south and east.

The fact that more house flies were recovered in the east traps than in the west is not quite in accord with the idea that they may have traveled largely with the wind, since there was no west wind but a considerable amount of east wind. With *Chrysomya macellaria*, however, there were less than a third as many in the east traps as in the west, the north traps caught the greatest number, and the south traps less than one-fourth as many as were caught in the north. The fact that the wind was more or less choppy throughout the period makes it very difficult to draw any conclusions on its exact influence on dispersion. Numerous other factors which must be considered also tend to make the drawing of conclusions on this point hazardous.

If we attempted to account for the greater catch of flies in certain lines of traps over others by the traffic along the highways we would expect to find the greatest dispersion east and west, probably greater to the west, and about equal to the north and south, but such was not the case.

Considering the possibility of the attraction of feeding and breeding places or volume of odors, we would expect, at least for *Musca domestica*, the greatest movement westward toward the city of Dallas, but the figures do not accord with this idea. It is probable that the proximity of smaller centers of attraction, such as farmhouses, barns, etc., are more effective in influencing the spread than more distant but larger attractive areas. These conditions, however, were apparently quite similar in each direction. As *Chrysomya macellaria* and *Phormia regina* are

normally nondomestic, we would not expect the same distribution of these species as of the house fly, yet the proportions caught in different directions are very similar.

TABLE III.—*Climatological data relating to first and second dispersion tests at Dallas, Tex.*

Date.	Temperature.			Wind.		Rain.	Humidity.			Actual sun.
	Max.	Min.	Mean.	Direction during successive hours from 6 a. m. to 8 p. m.	Velocity during day.		Max.	Min.	Mean.	
1916.	° F.	° F.	° F.		Miles.	Inch.	Per cent.	Per cent.	Per cent.	Per cent.
June 29	89	70	80	4 SE., 10 E	7 to 14	0	87	55	71	100
30	93	74	83.5	10 S., 4 E	10 to 13	0	85	47	66	88
July 1	93	70	81.5	3 SE., 3 S, 2 SW., 2 E., 4 SE	4 to 10	0	84	40	62	97
2	92	73	82.5	2 E., 12 SE	2 to 8	0	80	48	64	72
3	93	72	82.5	2 SE., 3 S., 4 SE., 5 E	3 to 13	0	78	44	61	90
4	94	73	83.5	2 E., 1 S., 1 SW., 5 E., 2 SW., 3 E.	2 to 15	.07	83	47	65	67
5	92	74	83.0	3 N., 8 NE., 3 N	5 to 14	0	77	49	63	73
6	93	74	83.5	2 NW., 3 N., 6 NW., 3 N	4 to 10	0	68	36	52	86
7	96	75	85.5	3 W., 2 N., 4 NW., 5 N	2 to 12	0	78	45	61	97
8	96	73	84.5	2 N., 5 E., 5 N., 2 NE	2 to 11	0	72	36	54	93
9	96	73	84.5	6 NW., 3 W., 5 NE	3 to 10	0	74	39	51	89
10	99	74	86.5	2 NW., 4 SE., 5 W., 3 E	2 to 7	0	74	24	49	100
11	98	77	87.5	2 SE., 4 SW., 5 SE., 3 S	2 to 11	T	69	30	49	86
12	95	78	86.5	13 S., 1 SE	5 to 13	0	68	34	51	78
13	95	77	86.0	2 SE., 12 S	4 to 11	0	63	41	52	71
14	95	78	86.5	2 S., 3 SW., 2 S., 7 SE	5 to 11	0	64	38	51	73
15	97	77	87.0	2 SW., 3 W., 1 S., 8 SE	3 to 7	0	68	43	55	89
16	101	77	89.0	2 NW., 3 W., 4 S., 5 SE	4 to 14	0	62	33	47	74
17	97	75	86.0	2 E., 1 SE., 1 S., 10 E	2 to 11	0	85	48	66	79
18	98	77	87.5	1 E., 6 SE., 1 S., 6 SE	4 to 10	0	85	35	60	89
19	99	76	87.5	4 SE., 3 E., 1 SE., 6 E	5 to 14	0	80	36	58	84
20	95	76	85.5	3 NE., 5 NW., 3 W., 3 N	5 to 12	T	80	49	64	60
21	95	76	85.5	5 NE., 1 E., 3 N., 5 NE	5 to 11	T	79	50	64	65
22	97	75	86.0	3 NW., 1 SW., 2 N., 8 NE	3 to 11	0	78	38	58	100
23	97	79	88.0	9 E., 5 NE	3 to 11	0	76	48	62	99
24	95	76	85.5	2 NE., 3 E., 9 NE	4 to 13	0	60	39	49	94
25	96	76	86.0	1 NE., 6 E., 1 NE., 6 E	5 to 17	0	60	31	45	88
26	88	72	80.0	9 E., 1 SE., 2 E., 2 SE	4 to 17	.19	98	85	91	43

RELATION OF SEX TO DISPERSION

Unfortunately the percentage of males and females among the flies liberated was not determined, hence no information can be gained from this test as to the relative proportion of the sexes among the flies recovered. It will be noted (Table II) that in the first ring of traps about the point of liberation 88 per cent of the marked house flies taken were females and 63.2 per cent of the screw-worm flies were females. In the second ring of traps the percentages of females of these two species were 92.8 per cent and 70.7 per cent, respectively; in the third ring 92.8 per cent and 78.3 per cent, and in the fourth ring 90.4 per cent and 75.5 per cent. So few marked flies of the other species were recovered that the sex data on them will not be discussed separately. It is noted that among the 4,652 marked flies recovered 3,952 were females, or 84.9 per cent. It might be supposed that if dissemination is induced largely by instinctive search for food and places to oviposit the females might tend to travel farther than the males, but the foregoing figures hardly justify

this conclusion, especially in the case of the house fly, which showed a fairly uniform relation between the number of males and females caught in the different circles of traps. Among the screw-worm flies the females appear to have shown a slightly more marked tendency toward distant dispersion.

RAPIDITY OF DISPERSION

In this test it was not possible to get very accurate data on the rapidity with which the different traps were reached, owing to the fact that the traps were not examined at frequent enough intervals to get accurate figures. A number of specimens of house flies were recovered in every trap, including the most remote ones, the day following liberation when the first examination was made, and the vast majority of all marked flies recovered had entered the traps at the end of the fourth day. Several specimens were recovered in nearly all of the traps up to the eighth day, and a few specimens were recovered after this, the last being taken on the twelfth day. It is possible that some may have been recovered after this, but as a second liberation was made on that date it would be impossible to determine whether the flies belonged to the first or second lot. Computation of the percentage of the flies recovered on different days in the different circles of traps indicates that the marked flies reached the nearer traps somewhat sooner than the more distant ones.

SECOND DISPERSION TEST AT DALLAS, TEX., 1916

On July 11, 1916, at 12.45 p. m., about 8,000 flies, marked with red chalk, were liberated at the same point as in the previous experiments. These consisted of about 70 per cent *Musca domestica*, 25 per cent *Chrysomya macellaria*, 4 per cent *Phormia regina*, 1 per cent *Lucilia sericata*, *Ophyra* spp. and *Sarcophaga* spp., etc. On July 12, during the forenoon, a second liberation of about 25,000 marked flies was made. This lot was estimated at about 50 per cent *M. domestica*, 40 per cent *C. macellaria*, 9 per cent *P. regina*, and 1 per cent *L. sericata*, *Sarcophaga* spp., etc. Part of these flies were colored with paint pigment known as "fast red" and others with powdered chalk. At the time of release a 7- or 8-mile breeze was blowing from the south, the sky was clear, and the sun was very hot.

In this test the traps in the first and second circles of the previous experiment were moved beyond the outer circle in the previous experiment, the traps in circles 3 and 4 in the previous experiment becoming numbers 1 and 2 in the present test. The environment of these eight traps has been mentioned in the discussion of the previous experiment. The positions of the outer rings of traps are briefly described as follows: West, No. 3, by farm lot in trees, stable and house 50 yards distant; No. 4, by grocery store in edge of city of Dallas. North, No. 3, in edge of cornfield ⅓ mile north of the village of Reinhardt; No. 4, in cornfield

⅛ mile from nearest farmhouse. East, No. 3, in weeds 200 yards east of a farmhouse; No. 4, 100 yards from farmhouse and pens where much live stock is kept. South, No. 3, under tree by small grocery store at cross-roads; No. 4, in village of Elam near stores and house. The distances from the release point to the traps are given in Table IV.

TABLE IV.—*Second dispersion test at Dallas, Tex. Species and sex of marked flies recovered in rings of traps about point of liberation*

Trap No.	Distance from place of release.	*Musca domestica.*		*Chrysomya macellaria.*		*Phormia regina.*		*Ophyra leucostoma.*		*Sarcophaga.* spp.		Undetermined.		Total.		Grand total.
	Miles.	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
1 W	1.6	0	33	10	28	0	5	0	1	0	0	0	0	10	67	77
1 N	1.8	0	8	3	18	0	0	0	0	0	0	0	0	3	26	29
1 E	2.0	0	10	8	19	0	2	0	0	0	0	0	0	8	31	39
1 S	2.0	0	4	1	3	0	0	0	1	0	0	0	0	1	8	9
Total		0	55	22	68	0	7	0	2	0	0	0	0	22	132	154
2 W	2.5	0	23	8	18	0	2	0	0	0	0	0	1	8	44	52
2 N	2.4	0	29	9	13	0	0	0	1	0	1	0	0	9	44	53
2 E	3.5	0	8	1	5	0	0	0	0	0	0	0	0	1	13	14
2 S	2.9	0	1	1	0	0	1	0	0	0	0	0	0	1	2	3
Total		0	61	19	36	0	3	0	1	0	1	0	1	19	103	122
3 W	3.0	0	5	6	25	2	10	0	2	0	1	0	0	8	43	51
3 N	3.4	0	4	4	18	0	2	0	[a]1	0	0	0	0	4	25	29
3 E	4.2	0	1	1	4	0	0	0	0	0	0	0	0	1	5	6
3 S	4.0	0	3	2	6	0	0	0	0	0	0	0	0	2	9	11
Total		0	13	13	53	2	12	[illegible]	3	0	1	0	0	15	82	97
4 W	3.75	0	6	0	9	0	1	0	1	0	0	0	0	0	17	17
4 N	4.4	0	9	11	35	0	0	0	0	0	0	0	0	11	44	55
4 E	5.0	0	2	3	5	0	0	0	0	0	0	0	0	3	7	10
4 S	5.0	0	0	0	2	0	1	0	0	0	0	0	0	0	3	3
Total		0	17	14	51	0	2	0	1	0	0	0	0	14	71	85
Grand total		0	146	68	208	2	24	0	7	0	2	0	1	70	388	458

[a] *Ophyra aenescens* Wied.

Since a single fly liberated at the beginning of the first test was recovered on July 11, it is barely possible that other marked specimens liberated at that time were recovered after July 12. In this discussion they would be considered as liberated in the second series.

In this experiment the number of colored flies captured was very much less than the number caught in the first test beginning June 30. The total number of marked flies recovered in the different directions from the point of liberation was 458, as compared with 4,652 in the first test. It is true that almost one-third more flies were liberated in the first test than in this one, but the proportion of those recovered is much smaller. It is also true that two of the rings of traps were much more distant from the point of liberation than in the first test; however, when we compare the catches in the two inner circles of traps in this test with the two outer ones in the first—positions which were the same in the two experiments—

we find that the percentage of marked flies recovered as compared with those liberated was 0.08 and 0.16, respectively (Tables II and IV).

Another factor which should have reduced the proportion of the marked flies recovered in these two rings of traps in the first test was that many were captured in the two circles of traps nearer the point of release. This difference in the number of marked flies recovered in the two experiments seems to be chargeable logically to climatic conditions. On July 11 and the following days the temperature ran markedly higher than during the period immediately after the first liberation, and the humidity ran correspondingly lower. This would have the effect of reducing the longevity, and possibly causing flies to seek shelter and moisture rather than to disperse freely.

The proportions of the several species among the marked flies released (estimated) and recovered, respectively, were as follows: *Musca domestica*, 52 per cent and 32 per cent; *Chrysomya macellaria*, 36 per cent and 60 per cent; *Phormia regina*, 8 per cent and 6 per cent; other species 0.01 per cent and 1.53 per cent. The hot weather was probably a greater inhibiting factor in the case of the house fly and *P. regina* than in that of *C. macellaria*. The optimum temperature is higher in the case of the last species. The make-up of the total catch in the recovery traps was not determined, but it was evident that there was a marked decrease in the total catch toward the end of the period and a marked reduction in the number of *P. regina* captured.

DISTANCE OF DISPERSION

Numerous marked specimens of *Musca domestica* and *Chrysomya macellaria* reached the more distant ring of traps, several specimens of both species being recovered in trap No. 4 east, 5 miles from the point of liberation, and three specimens of *C. macellaria* in No. 4, 5 miles south. Not a single *M. domestica* was recovered in No. 4 south, however. *Phormia regina* also reached the outside ring of traps, one specimen being taken in No. 4 south (5 miles), one in No. 4 west (3.75 miles), and many in No. 3 west (3 miles). One specimen of *Ophyra leucostoma* Wied. was recovered in No. 4 west (3.75 miles) and one specimen of *O. aenescens* Wied. in No. 3 north (3.4 miles). Only two specimens of *Sarcophaga* spp. were recovered, one in No. 3 west (3 miles) and one in No. 2 north (2.4 miles). The number of flies recovered at different distances is shown in detail in Table IV.

DIRECTION OF SPREAD

The individual species should be treated separately, but the several species recovered in the different directions seem to remain in about the same proportion with the exception of *Phormia regina*, which showed a marked tendency to go westward, 20 specimens (77 per cent) being taken in the west line of traps, while only two were taken in the traps in

each of the other directions. The large number of marked flies taken in the lines of traps west and north as compared with those taken in the traps east and south strongly indicates that there was a tendency in this experiment also to travel with the wind, as the prevailing wind during the first nine days was from the south to east, and during the first five days the wind was almost constantly from the south and southeast. The respective number and percentage of house flies and screw-worm flies recovered in the different directions were as follows (based on total number of marked specimens of each species): West, 67 (45.89 per cent) and 104 (37.68 per cent); north, 50 (34.25 per cent) and 111 (40.22 per cent); east, 21 (14.38 per cent) and 46 (16.67 per cent); south, 8 (5.48 per cent) and 15 (5.43 per cent). All but one of the specimens of Ophyra and Sarcophaga were recovered in the west and north traps.

The influence of traffic along the roads on dispersion would be practically the same as in the first test; that is, the principal traffic was east and west of the point of liberation. Heavy traffic also passed between No. 3 and 4 north and 3 and 4 south.

SEX IN RELATION TO MIGRATORY TENDENCY

In this test it is noteworthy that not a single male house fly was recovered. With the screw-worm fly the percentage of females recovered was 75.3. This percentage was remarkably uniform in the different directions from the point of liberation, ranging from 71.7 on the east line of traps to 76.9 on the west line. Considering the distance of dispersion of the males and females of the screw worm there is a slight indication that there was a greater tendency toward wide dispersion among the females than among the males. It may be worthy of note that the females appeared to enter the various traps in greater numbers during the early part of the recovery period than did the males. For instance, the percentage of males entering all of the traps during the first three days of the test was about 21, while during the remainder of the recovery period it was 45. This should not be construed as meaning that the males were necessarily slower in dispersion, but they may have displayed less eagerness to find food and hence to become entrapped.

RAPIDITY OF DISPERSION

Comparatively few marked flies were recovered in the traps when emptied the first day after liberation. This was especially notable in the fourth ring of traps (3.75 to 5 miles from the point of liberation) where only a single specimen of *Chrysomya macellaria* was recovered. The recoveries the second day (July 13) were markedly larger than the first, this day giving the maximum daily catch (134) of *C. macellaria* for the period. This species seemed on this day to be generally distributed throughout the area, nearly all traps showing a marked decline in the

number captured after this date. The house flies appear to have become widely disseminated by the end of the second day, and the maximum number was recovered on the fourth day (July 15). This maximum, however, was not attained through large numbers being captured in the more distant traps but by an increased catch in the two inner rings. The trapping was continued until July 26, the last *C. macellaria* being captured on the eleventh day (July 22), one specimen appearing on that date in each of traps No. 4 north and No. 4 south. The last house flies recovered were taken on the same date, one each in No. 1 south and No. 3 south. In No. 2 north 7 house flies were recovered on July 21, a much larger proportion on this late date than was observed in any other trap. The total in this trap was 29.

Several *Phormia regina* out of a total of 26 recovered were taken soon after liberation, one appearing in No. 2 west and three in No. 3 west on the first day. The last *P. regina* was recovered on the tenth day (July 21) in No. 3 west.

There were too few specimens of Ophyra liberated or recovered to draw conclusions, but it may be stated that the first specimens recovered were taken in No. 3 west, No. 2 north, and No. 1 south (one in each) on the third day after liberation and the last in No. 2 west on the seventh day (July 18).

THIRD DISPERSION TEST AT DALLAS, TEX.

The evidence secured in the second test very clearly indicated that the maximum distance of normal dispersion of the various species of flies was far greater than the outer circle of traps in that test, which was approximately 5 miles. Accordingly plans were laid for another test to determine if possible the maximum distance of dissemination. For this test the region lying to the north of Dallas was chosen. The point of liberation selected was approximately 7 miles from the edge of the city of Dallas on the main north and south thoroughfare known as "Kings Highway." This region is highly developed agriculturally, being thickly dotted in all directions with farms, most of which maintain some live stock. The contour of the country is not markedly different from that where the first and second tests east of Dallas were conducted. Within the area under consideration are a number of creeks bordered with woods of greater or less width. For the most part, however, the country is open cultivated land more or less closely covered with a network of roads, several steam railways, and one electric line. As has been stated, the point of liberation was on the most heavily traveled highway running north from Dallas. On this road, about 4 miles south of the point of liberation, is the town of Vickery (about 100 population) and to the north 1 mile the town of Richardson (500 population), Plano (1,700 population) 6½ miles, and Allen (300 population) 13 miles. To the east are a number

of small community centers within a radius of 4 miles from the point of liberation and the towns of Garland (1,400 population) 6 miles, Rowlett (150 population) 10 miles, and Rockwall (1,400 population) 16 miles. To the west are also several community centers and the towns of Farmers Branch (250 population) 9½ miles and Carrollton (575 population) 10 miles.

Fairly direct though not heavily traveled roads lead east and west from near the point of liberation. The traffic on the east road outward from a corner 5½ miles east of the point of release is heavy, being probably equal to that north and south of the point of release. The traps were set along these highways where they could be examined and rebaited expeditiously.

The principal roads, location of towns and main streams, and their relation to the point of release and the recovery traps are indicated on the accompanying map (fig. 2). It will be noted that the north line of traps bears somewhat to the east of due north from the point of release and those to the south are about 2 miles west of south, while the east traps were about 1 mile south of east and those to the west practically due west. Sixteen recovery traps were utilized, four being set in each direction from the release point. These were 18-inch cone traps. The immediate environment of the traps may be described briefly as follows: West, No. 1, 4½ miles, under shed in cotton field, no stock near except chickens; No. 2, 6 miles, adjacent to hogpen and near mule barn on farm; No. 3, 7 miles, under shed by mule barn, cow lot, and hogpen; No. 4, 8.1 miles, by crib next to hogpen and cow lot. North, No. 1, 4.1 miles, under hedge near hogpen; No. 2, 5.6 miles, in old hogpen near barn, mules and chickens kept; No. 3, 6.3 miles, under seed house at gin in edge of town; No. 4, 7.8 miles, under hedge in cotton field, no stock on farm. East, No. 1, 4.7 miles, under empty corn crib, no stock near; No. 2, 6.2 miles, under water tank by horse barn; No. 3, 7.2 miles, adjacent to hogpen and mule barn; No. 4, 8 miles, in vacant shed one-third mile from main road and 100 yards from stock barns. South, No. 1, 4.6 miles, near house in edge of village, no live stock near; No. 2, 6.1 miles, adjacent to house, barns, and hogpen; No. 3, 7.3 miles, in shed near yards with all classes of live stock; No. 4, 8.2 miles, under tree in edge of city.

On September 17, the day prior to liberation, all traps were baited with fresh gut slime, and they were all rebaited on September 23 and 27 and October 3. Beginning with September 20, and continuing until September 28, the flies were killed and examined daily in all traps. The traps were not emptied on September 29, October 1, 2, and 4 (except No. 1 and 2 south). October 5 was the last date when all traps were emptied and the last date upon which any marked flies were recovered. On October 7 all flies in traps 2 and 3 south, and on October 8 all flies in traps 4 west and 4 south were killed and examined, but no marked flies were found.

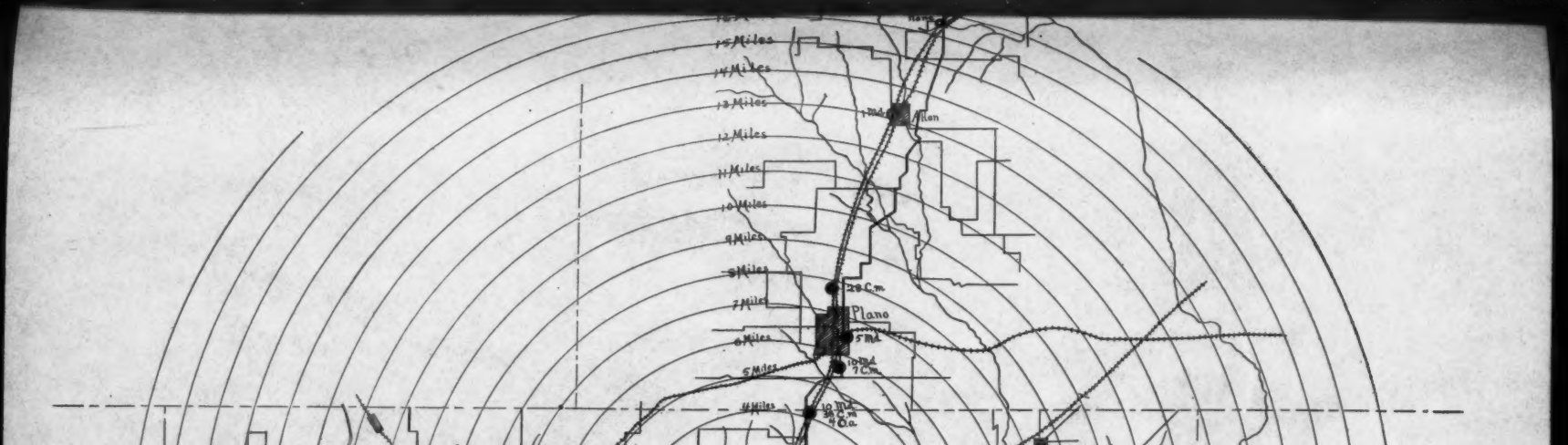

15 Miles
14 Miles
13 Miles
12 Miles
11 Miles
10 Miles
9 Miles
8 Miles
7 Miles
6 Miles
5 Miles
4 Miles
Allen
Plano
20 C.m.
5 Mi.
10 Mi.
70 C.m.
10 Mi.
90 C.m.
90 a

FIG. 2.—Map showing location of third and fourth dispersion tests at Dallas, Tex. Dots indicate location of traps in third test and rings location of traps in fourth test. Circles indicate distances from point of release.

50937°—21. (To face page 746.)

On September 18, between 10 and 11 a. m., about 20,000 marked flies were liberated, and between 3 and 4 p. m., 40,000 were released. These flies were secured by means of traps at an abattoir in Dallas, the first lot being taken between noon of the previous day and 9 o'clock on the morning of release and the second lot between 9 a. m. and 2 p. m. on the same date. The flies were transported to the point of release in screened cylinders containing green branches. They were then well colored with powdered red crayon by placing the cylinders in a canvas bag. When liberated the flies appeared to be very hungry, and many of them clung to our clothing and bodies in an effort to secure moisture, but the vast majority flew into the air, some apparently going up 20 or 30 feet and disappearing in all directions, but seemingly more going northward than in other directions. At the time of release the sky was clear and a gentle breeze was blowing from the south. The maximum temperature for the day was 95° F. All flies were brushed off clothing and cylinders, and two stops were made after the scene of release was left in order to make sure that no colored flies were following.

The proportions of the different species released were about as follows: *Musca domestica* 42.32 per cent, *Chrysomya macellaria* 54.90 per cent, *Phormia regina* 1.25 per cent, *Sarcophaga* spp. 1.25 per cent, *Lucilia sericata* 0.14 per cent, *Ophyra leucostoma* and *O. aenescens* 0.14 per cent. The approximate averages of the species in the entire catch in the 16 traps were: *Musca domestica* 44 per cent, *C. macellaria* 44 per cent, *P. regina* 1 per cent, *Ophyra* spp. 0.06 per cent, *Lucilia* spp. 1 per cent, *Sarcophaga* spp. 8 per cent.

The proportion of the species among the marked specimens recovered was *Musca domestica* 20 per cent, *Chrysomya macellaria* 78.84 per cent, *Ophyra aenescens* 1.16 per cent. No marked specimens of *Phormia regina, Lucilia sericata*, or *Sarcophaga* spp. were captured. Comparing these percentages with the percentages of the species released, we note that the percentage of Ophyra recovered is over eight times, *C. macellaria* slightly less than one and one-half times, and *M. domestica* less than one-half the percentage released.

DISTANCE OF DISPERSION

As is shown in Table V, the number of marked house flies reaching the four concentric rings of traps from the center outward was 30, 23, 11, and 5, a total of 69. Expressing this in percentages, we have 43.5, 33.3, 15.9, and 7.3. The maximum distance recorded for this species in this test was 8.1 miles in trap No. 4 west, where three marked flies were recovered. This was almost equaled in trap No. 4 east (7.9 miles) where two colored house flies were recovered.

TABLE V.—*Third dispersion test at Dallas, Tex. Species and sex of marked flies recovered in rings of traps about point of liberation*

Trap No.	Distance from place of release.	*Musca domestica.*				*Chrysomya macellaria.*				*Ophyra aenescens.*				Total.				Grand total (number).
		Number.		Per cent.		Number.		Per cent.		Number.		Per cent.		Number.		Per cent.		
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
	Miles.																	
1 W....	4.5	1	2	33.3	66.7	5	43	10.4	89.6	0	0	0	0	6	45	11.8	88.2	51
1 N.....	4.1	0	15	00.0	100.0	6	32	16.8	84.2	2	2	50	50	8	49	14.04	85.96	57
1 E.....	4.7	0	2	00.0	100.0	8	23	25.8	74.2	0	0	0	0	8	25	24.2	75.8	33
1 S.....	4.6	1	9	10.0	90.0	4	34	10.5	89.5	0	0	0	0	5	43	10.4	89.6	48
Total...		2	28	6.67	93.33	23	132	14.8	85.2	2	2	50	50	27	162	14.29	85.71	189
2 W....	6.0	0	1	.0	100.0	1	20	4.8	95.2	0	0	.....	.....	1	21	4.5	95.5	22
2 N.....	5.6	3	10	23.08	76.92	0	7	.0	100.0	0	0	.....	.....	3	17	15.0	85.0	20
2 E.....	6.2	0	0	.....	.....	0	13	.0	100.0	0	0	.....	.....	0	13	.0	100.0	13
2 S.....	6.1	1	8	11.1	88.9	3	10	23.1	76.9	0	0	.....	.....	4	18	18.2	81.8	22
Total...		4	19	17.39	82.61	4	50	7.4	92.6	0	0	.....	.....	8	69	10.39	89.61	77
3 W....	7.0	0	2	.0	100.0	0	4	.0	100.0	0	0	.....	.....	0	6	.0	100.00	6
3 N.....	6.3	2	3	40.0	60.0	0	0	.....	.....	0	0	.....	.....	2	3	40.0	60.0	5
3 E.....	7.2	1	3	25.0	75.0	0	6	.0	100.0	0	0	.....	.....	1	9	10.0	90.0	10
3 S.....	7.3	0	0	.....	.....	0	3	.0	100.0	0	0	.....	.....	0	3	.0	100.0	3
Total...		3	8	27.3	72.7	0	13	.0	100.0	0	0	.....	.....	3	21	12.5	87.5	24
4 W....	8.1	0	3	.0	100.0	0	8	.0	100.0	0	0	.....	.....	0	11	.0	100.0	11
4 N.....	7.8	0	0	.....	.....	3	25	10.7	89.3	0	0	.....	.....	3	25	10.7	89.3	28
4 E.....	8.0	0	2	.0	100.0	0	5	0.0	100.0	0	0	.....	.....	0	7	.0	100.0	7
4 S.....	8.2	0	0	.....	.....	2	7	22.2	77.8	0	0	.....	.....	2	7	22.2	77.8	9
Total...		0	5	.0	100.0	5	45	10.0	90.0	0	0	.....	.....	5	50	9.1	90.9	55
Grand total..		9	60	13.04	86.96	32	240	11.76	88.24	2	2	50	50	43	302	12.44	87.56	345

As will be noted, a considerably larger number of marked *Chrysomya macellaria* were recovered, the total being 272. Of these, 155 (57 per cent) were taken in the first ring of traps, 54 (19.8 per cent) in the second ring, 13 (4.8 per cent) in the third ring, and 50 (18.4 per cent) in the fourth ring. It will be noted that a considerable number of marked flies of this species were taken in each of the traps in the outer ring, which is approximately 8 miles from the point of liberation, the maximum distance being 8.2 miles in No. 4 south, where 9 colored flies were recovered. The comparatively small number of marked screw-worm flies taken in the third ring of traps is noteworthy, but this can be explained largely on the basis of the comparatively unfavorable location of these traps for the collection of this species. This matter will be discussed subsequently.

The only other species of marked flies captured was *Ophyra aenescens*, four specimens of which were taken in No. 1 north, 4.4 miles from the point of liberation.

TABLE VI.—*Third dispersion test at Dallas, Tex. Species and sex of marked flies recovered in different directions from point of liberation*

Trap No.	Distance from place of release.	*Musca domestica.*				*Chrysomya macellaria.*				*Ophyra aenescens.*				Total.				Grand total (number).
		Number.		Per cent.		Number.		Per cent.		Number.		Per cent.		Number.		Per cent.		
	Miles.	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
1 W....	4.5	1	2	33	67.0	5	43	10.4	89.6	0	0	.....	.....	6	45	11.8	88.2	51
2 W....	6.0	0	1	00	100.0	1	20	4.8	95.2	0	0	.....	.....	1	21	4.5	95.5	22
3 W....	7.0	0	2	00	100.0	0	4	0	100.0	0	0	.....	.....	0	6	0	100.0	6
4 W....	8.1	0	3	00	100.0	0	8	0	100.0	0	0	.....	.....	0	11	0	100.0	11
Total...		1	8	11.2	88.8	6	75	7.4	92.6	0	0	.....	.....	7	83	7.8	92.2	90
1 N....	4.1	0	15	00	100.0	6	32	15.8	84.2	2	2	50	50	8	49	14.04	85.96	57
2 N....	5.6	3	10	23	77.0	0	7	0	100.0	0	0	.....	.....	3	17	15.0	85.0	20
3 N....	6.3	2	3	40	60.0	0	0	.....	.....	0	0	.....	.....	2	3	40.0	60.0	5
4 N....	7.8	0	0	.....	.....	3	25	10.7	89.3	0	0	.....	.....	3	25	10.7	89.3	28
Total...		5	28	15.15	84.85	9	64	12.3	87.7	2	2	50	50	16	94	14.55	85.45	110
1 E.....	4.7	0	2	00	100.0	8	23	25.9	74.1	0	0	.....	.....	8	25	24.2	75.8	33
2 E.....	6.2	0	0	.....	.....	0	13	0	100.0	0	0	.....	.....	0	13	0	100.0	13
3 E.....	7.2	1	3	25	75.0	0	6	0	100.0	0	0	.....	.....	1	9	10.0	90.0	10
4 E	8.0	0	2	00	100.0	0	5	0	100.0	0	0	.....	.....	0	7	0	100.0	7
Total...		1	7	12.5	87.5	8	47	14.5	85.5	0	0	.....	.....	9	54	14.3	85.7	63
1 S.....	4.6	1	9	10.0	90.0	4	34	10.6	89.4	0	0	.....	.....	5	43	10.4	89.6	48
2 S.....	6.1	1	8	11.2	88.8	3	10	23.1	76.9	0	0	.....	.....	4	18	18.2	81.8	22
3 S.....	7.3	0	0	.....	.....	0	3	0	100.0	0	0	.....	.....	0	3	0	100.0	3
4 S.....	8.2	0	0	.....	.....	2	7	22.3	77.7	0	0	.....	.....	2	7	22.3	77.7	9
Total...		2	17	10.6	89.4	9	54	14.3	85.7	0	0	.....	.....	11	71	13.4	86.6	82
Grand total..		9	60	13.04	86.96	32	240	11.76	88.24	2	2	50	50	43	302	12.44	87.56	345

DIRECTION OF FLIGHT

In Table VI the numbers of marked flies recovered in the different directions from the point of liberation are set forth. In considering the house fly from the standpoint of the direction of travel as indicated by the number of marked specimens recovered, probably the most striking fact is that 33, or 47.8 per cent, of the marked flies were taken in the four traps to the north of the point of release, and the next largest number—19, or 27.5 per cent—were recovered in the traps in the opposite direction. The catches to the west and east were almost identical, the west with 9 flies, or 13 per cent, and the east with 8 flies, or 11.6 per cent.

In considering the factors which might influence dissemination of the house fly we immediately think of the attraction of feeding and breeding places, the character and amount of travel along the highways, and the direction and velocity of the wind. The attraction of odors from towns and cities would tend to favor the migration to the north and south, since there are several towns located along the north line of traps and a few to the south and east. If the flies are inclined to go toward odors borne a considerable distance on the wind, one would expect a greater number to the south, since the volume of odors from the city of Dallas

would be stronger than that originating elsewhere. If the attraction exerted is of more limited range, so that the proximity of farm buildings and small towns would exert the greater influence, we would probably find the greater number of specimens to the north, east, and south, which agrees fairly well with the facts.

TABLE VII.—*Climatological data relating to third dispersion test at Dallas, Tex.*

Date.	Temperature.			Wind.		Rain.	Humidity.			Actual sun.
	Max.	Min.	Mean.	Direction during successive hours from 6 a. m. to 8 p. m.	Velocity during day.		Max.	Min.	Mean.	
1918.	°F.	°F.	°F.		Miles.	Inch.	P. ct.	P. ct.	P. ct.	P. ct.
Sept. 18...	91	66	78.5	2 E., 2 SE., 8 S., 2 SE.	5 to 10	0	78	36	57	80
19...	79	62	70.5	1 S., 5 NE., 6 E., 2 NW.	5 to 21	0.57	65	56	60	34
20...	68	55	61.5	14 N.	7 to 13	0	72	55	63	70
21...	70	47	58.5	2 N., 6 E., 2 NE., 4 E.	1 to 9	0	83	34	58	100
22...	76	51	63.5	2 E., 11 SE., 1 S.	6 to 9	0	67	33	50	99
23...	80	53	66.5	2 E., 12 SE.	4 to 12	0	61	25	43	99
24...	85	55	70.0	6 SE., 1 S., 4 SE., 3 S.	7 to 17	0	68	45	56	91
25...	87	60	73.5	5 SE., 6 S., 3 SE.	10 to 20	0	85	52	68	78
26...	72	63	67.5	3 E., 3 N., 1 NE., 7 NW.	4 to 11	.86	83	69	76	25
27...	72	55	63.5	4 N., 1 NW., 9 N.	4 to 14	0	78	42	60	75
28...	75	48	61.5	12 NW., 1 W., 1 NW.	4 to 9	0	87	35	61	100
29...	85	51	68.0	6 W., 3 NW., 2 NE., 3 E.	2 to 7	0	73	29	51	99
30...	88	60	74.0	5 W., 1 SW., 4 S., 4 E.	3 to 7	0	61	23	42	100
Oct. 1...	89	62	75.5	4 E., 7 S., 3 SE.	1 to 10	0	62	27	44	93
2...	87	61	74.0	3 SE., 4 W., 5 SE., 2 E.	2 to 7	0	66	33	49	93
3...	89	63	76.0	3 E., 1 S., 3 SE., 7 S.	2 to 7	0	61	29	45	98
4...	89	66	77.5	2 E., 12 SE.	5 to 9	0	58	32	45	100
5...	89	67	78.0	3 S., 2 SW., 7 S., 2 SE.	3 to 13	0	70	38	54	62

As will be seen by referring to Table VII, the period during which this test was conducted was extremely unfavorable for determining the possible influence of wind on the dissemination of flies. During the entire period of recovery there was no considerable number of consecutive days in which the wind was constant. In other words, the wind condition would be expressed as "choppy," and with its continual varying from one quarter to another it is almost impossible for one to weigh the wind influence on dissemination in this test. For 7 hours following the liberation of the first lot of flies there was blowing a 5- to 10-mile breeze from the south. It is conceivable that the flies when liberated drifted rapidly northward with the wind and that many were subsequently carried back and in other directions with the wind changes. The assumption that they traveled with the wind is hardly borne out by the recovery of several flies in the south traps on the next day (Table VIII) and by the recovery of four flies in the east traps on September 22, where they must have gone at right angles to or against the wind. Moreover, there is strong evidence, as indicated by the collections of marked house flies in the four traps to the west of the point of liberation, that there is no pronounced tendency for them to travel with the wind, or at least that tendency is easily overcome by other influencing factors. During the first 5 days of the test not a single marked house fly was recovered in the west traps, and during this period the prevailing wind during the hours

of fly activity was from the east and southeast, 44 hours of wind being from those directions while the wind came from other directions for 37 hours, 16 of these being from the north and 7 from the northeast while no west or southwest wind whatever was experienced. The fact that all the flies captured in the west line of traps were taken on two days, September 24 and 25, 5 on the former and 4 on the latter, is peculiar, but no explanation is apparent. The prevailing wind on these two days was from the southeast and south and on the two days preceding from the southeast, while during the days preceding September 22 and succeeding September 25 no southwest wind was experienced during the period when marked house flies were being recovered.

The question of the amount of traffic along the highways was mentioned in the introductory remarks on this flight experiment. It is certainly true that there is far more traffic along the highway running north and south from the point of liberation than to the east and west, and this corresponds with the greatest dissemination. There is, however, heavier traffic to the south of the point than there is to the north, but many less flies were recovered. There is a comparatively small amount of traffic which would induce house flies to congregate and follow loads over any of the roads. No doubt the most attractive carriers would be milk wagons going into Dallas and returning to the rural districts, and these are not very numerous on the highways to the north. Other slow-moving vehicles would consist mostly of loads of cottonseed hulls and meal, which are not attractive, and miscellaneous groceries and supplies which would not be considered especially attractive. No garbage or manure is hauled over the roads in any direction. The major part of the traffic consists of passenger-carrying automobiles and some motor trucks. At the time this test was being carried on there were many families with various kinds of household goods and food driving teams north and south on the main highway. These slow-moving covered wagons might readily serve as a means of transport for house flies for some distance.

The dissemination of *Chrysomya macellaria* in the different directions was much more uniform than that of the house fly. Of the total of 272 marked screw-worm flies recovered, 81, or 29.8 per cent, were recovered in the four traps to the west, 73, or 26.8 per cent, to the north, 55, or 20.2 per cent to the east, and 63, or 23.2 per cent, to the south. The screw-worm fly, which is not especially attracted by urban conditions or habitations and is not inclined to come to or be carried by vehicles unless loaded with meat or meat products, would not be expected to show the same tendencies in dissemination as the house fly, and this seems to be indicated by the number of marked flies recovered in the different directions. With choppy wind conditions it would be equally as hard to determine the wind influence on this species as on the house fly.

TABLE VIII.—*Third dispersion test at Dallas, Tex. Number of marked Musca domestica and Chrysomya macellaria captured on successive dates after liberation*

Trap No.	Sept. 19.		Sept. 20.		Sept. 21.		Sept. 22.		Sept. 23.		Sept. 24.		Sept. 25.		Sept. 26.		Sept. 27.		Sept. 28.		Sept. 30.		Oct. 3.		Oct. 5.		Total.	
	M. d.	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*	*M. d.*	*C. m.*
1 W	0	39	0	2	0	0	0	4	0	1	2	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	3	48
2 W	0	13	0	0	0	0	0	1	0	1	0	3	1	2	0	0	0	0	0	0	0	0	0	0	0	1	1	21
3 W	0	3	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	4
4 W	0	4	0	1	0	0	0	0	0	0	1	2	2	0	0	0	0	0	0	0	0	0	0	1	0	0	3	8
Total	0	59	0	3	0	0	0	5	0	2	5	5	4	3	0	1	0	0	0	1	0	0	0	1	0	1	9	81
1 N	5	25	1	4	1	0	0	2	0	0	3	6	4	0	1	1	0	0	0	0	0	0	0	0	0	0	15	38
2 N	3	4	2	0	2	0	0	1	2	1	0	1	2	0	2	0	0	0	0	0	0	0	0	0	0	0	13	7
3 N	1	0	1	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0
4 N	0	15	0	2	0	2	0	5	0	2	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	28
Total	9	44	4	6	3	2	0	8	3	3	5	8	6	0	3	1	0	0	0	0	0	1	0	0	0	0	33	73
1 E	1	22	1	1	0	0	0	1	0	0	0	6	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	31
2 E	0	9	0	1	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13
3 E	0	4	0	1	0	0	3	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	6
4 E	0	4	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	5
Total	1	39	1	4	0	0	4	3	0	0	1	8	1	1	0	0	0	0	0	0	0	0	0	0	0	0	8	55
1 S	3	16	0	5	1	1	0	8	4	5	1	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	9	38
2 S	2	5	1	4	0	0	1	1	1	0	2	1	1	0	0	1	1	0	0	0	0	0	1	0	0	1	10	13
3 S	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
4 S	0	2	0	2	0	0	0	1	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	9
Total	5	23	1	11	1	1	1	10	5	11	3	1	1	2	0	3	1	0	0	0	0	0	1	0	0	1	19	63
Grand total	15	165	6	24	4	3	5	26	8	16	14	22	12	6	3	5	1	0	0	1	0	1	1	1	0	2	69	272
Percentage [a]	21. 74	.....	8. 69	.....	5. 80	.....	7. 25	.....	11. 59	.....	20. 29	.....	17. 39	.....	4. 35	.....	1. 45	.....	0	.....	0. 37	.....	1. 45	.....	0	.....	100	.....
Percentage [b]	.....	60. 65	.....	8. 82	.....	1. 10	.....	9. 56	.....	5. 88	.....	8. 09	.....	2. 21	.....	1. 84	.....	0	.....	0. 37	.....	. 37	.....	. 37	.....	. 74	.....	100

[a] Percentage of marked *Musca domestica* caught each day based on total marked *M. domestica* recovered.
[b] Percentage of marked *Chrysomya macellaria* caught each day based on total marked *C. macellaria* recovered.

It is peculiar that all four of the specimens of *Ophyra aenescens* were taken in a single trap, No. 1 north. Little is known of the habits and responses to various stimuli of this species, but we usually think of it as being most abundant under rural conditions and in no sense a domestic species. These flies appear to be attracted to a considerable extent by carcasses in their last stages of decay and to some extent by hog manure.

RAPIDITY OF FLIGHT

Probably the best method of indicating the rapidity of dispersion from the point of release is by tabulating the number of marked specimens recovered in the various traps on succeeding days. These data are given in Table VIII. While it might appear that the house fly traveled very slowly to the west, none being recovered during the first 5 days, the fact that some marked specimens were taken in the most remote recovery trap in that direction on that date would indicate that this must be explained on some other basis than the time required to reach the traps. It will be noted that one marked specimen was taken in trap No. 3 north, 7.1 miles from the point of liberation, on the day following release, and two specimens were taken in trap No. 2 south, 6.1 miles, on the same day. The first specimen reaching No. 4 east, 7.9 miles, was on the fourth day after release. As no marked specimens were captured in traps No. 4 north and No. 3 and 4 south, any further statement as to the rapidity of dispersion to the outside of the 8-mile circle is hazardous. The fact that more marked flies were taken in the recovery traps on the first day than on any subsequent date after release indicates that spread of this species is rapid.

The large number of screw-worm flies recovered in all the traps on the first day after release indicates a very rapid and thorough dispersion of this species promptly after release. It is notable that more marked specimens were taken in the outside circle of traps, approximately 8 miles, on the first day following release than on any subsequent day. Of course the recoveries were affected markedly by temperatures as indicated by the comparatively few specimens taken on September 21 and 22 and the larger number taken September 24, which was a mild day. No doubt low temperatures would retard dissemination, but they would also have the effect of rendering the baits in the recovery traps less attractive and hence, even though the marked flies were in the vicinity, they might not be captured.

It would appear that in the case of neither the house fly nor the screw-worm fly were the recoveries any more gradual in the outer rings of traps than in the closer ones. In other words, there did not seem to be any percolation of the flies outward from the point of release. This, of course, would be influenced by the choppy wind condition already mentioned, provided the wind is a dominant factor in dispersion.

Three of the four specimens of *Ophyra aenescens* were taken on the third day following release and the other on the fourth day. This species, which is ordinarily thought of as having little power of flight, has shown rather remarkable powers of dissemination in this instance.

RELATION OF SEX TO DISPERSION

The proportion of the sexes of flies released, based on examination of a considerable number of specimens killed at the time of release, was: Males, *Musca domestica* 25.6 per cent, *Chrysomya macellaria* 15.6 per cent, *Phormia regina* none, *Lucilia sericata* none, *Ophyra* spp. none, *Sarcophaga* spp. 22.2 per cent. It will be noted by referring to Table V that the percentage of male *M. domestica* recovered (13) was not greatly different from the percentage (15.6) released. It is rather strange that one-half of the Ophyra recovered were males, while no males were noted in the material examined at the time of liberation.

Referring to *Musca domestica*, the proportion of males to females increased from the nearer circle of traps (6.7 per cent) outward to the third circle (27.3 per cent), but no males were caught in the outer circle. This does not indicate that this sex is more limited in distance of dispersion, since only 5 specimens were taken in the outer circle and the proportion of males to females was so small that the chance of recovering any among that number was meager. The maximum distance of flight of any male house fly was 7.2 miles, one specimen being recovered in No. 3 east. The proportion of sexes of this species in the different directions was about the same (Table VI), ranging from about 10.6 per cent males in the traps to the south to 15.2 per cent in the traps to the north.

In the case of *Chrysomya macellaria* the proportion of males to females varied considerably in the rings of traps at different distances, but not in a way to show any relation between sex and distance. The percentage of males in the first ring of traps (4.5 miles) was highest (14.8) and in the third ring lowest (none). The same erratic results are also apparent in the proportion of sexes in different directions, but the average percentage of males taken in the traps in the different directions is not very different, ranging from 7.4 in the west line of traps to 14.5 in the east line.

RELATION OF POSITION OF TRAPS TO THE RECOVERY OF MARKED FLIES

At the outset in these dispersion studies it became apparent that the environment of the recovery traps had a marked effect not only on the size of the catch but on the proportion of the species. In other words, certain situations occupied by recovery traps were preeminently house-fly situations while in others the blowflies dominated. As an illustration the results in the case of traps No. 3 and 4 north may be

cited. The first of these was in a village and most decidedly a house-fly environment, as indicated by a catch of 361 gm., of which 92 per cent were house flies. No. 4 was in a field removed from human habitations and hence more favorable for screw-worm flies. Here 78 per cent of the 548 gm. taken were screw-worm flies. Now note the number of marked flies recovered: Trap No. 3, 5 house flies and no screw-worm flies; trap No. 4, 28 screw-worm flies and no house flies.

It seems evident that those positions favorable for the capture of large numbers of one or more species of flies must be in the favorite haunts of

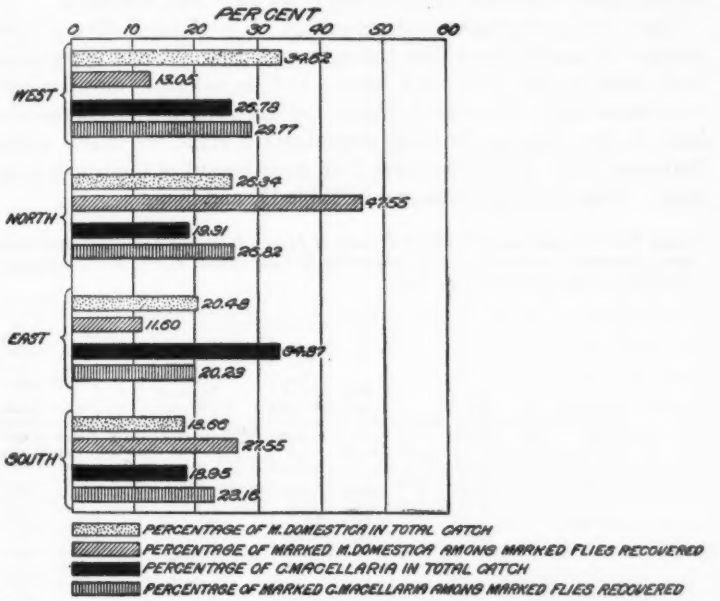


FIG. 3.—Diagram showing percentage of each species taken in each direction from point of liberation in the third dispersion test at Dallas, Tex. First, percentage of house flies in each direction as compared with total house flies caught; second, percentage of marked house flies as compared with the total marked house flies recovered; third, percentage of screw-worm flies in each direction as compared with total catch of this species; fourth, percentage of marked screw-worm flies as compared with the total marked screw-worm flies recovered.

the species so taken and that marked specimens in their movements would, like others, tend to congregate in such positions. Furthermore, it would appear that those traps so located as to catch the largest number of flies of a given species would have a better chance of capturing marked specimens. It is true that the occurrence of favorable breeding and feeding places in the immediate environs of a trap would favor large catches, but these very conditions would tend to assemble migrating marked individuals near the trap.

It was at first thought that by weighing the relative advantages of the positions of the traps in the capture of house flies and screw-worm flies

an index might be worked out and thus the difference in position equalized and the normal dispersion of the marked specimens be determined more accurately. This matter upon study became so complicated and so many unknown factors entered that it was dropped. It was finally concluded that a comparison of the relative positions might be made by computing in the form of percentage the relation between each of these principal species in the catches in the different traps and the total catch of each of the species in all the recovery traps. And again the percentage of marked flies of the two species in each trap as compared with the total marked flies of each species recovered in all traps was computed.

These two percentages—the percentage of total house flies and percentage of marked house flies taken in each trap—should approximate each other in size if all other factors such as distance, direction, etc., were eliminated. The same is true of the screw-worm fly. On the other hand, if the difference in these percentages is great, we must conclude that some other factor than mere local environment of the trap is dominant. These data are presented in Table IX and figure 3.

TABLE IX.—*Weight and proportion of catch of Musca domestica and Chrysomya macellaria compared with number and proportion of these species among marked specimens recovered in third test at Dallas, Tex.*

Trap No.	Total weight of *M. domestica* and *C. macellaria* caught.	Weight of *M. domestica* caught in trap.	Percentage of *M. domestica* compared with total in trap.	Weight of *C. macellaria* caught in trap.	Percentage of *C. macellaria* compared with total in trap.	Percentage of *M. domestica* in each trap compared with total *M. domestica* in all traps.	Number of marked *M. domestica* caught.	Percentage of marked *M. domestica* in each trap compared with total marked *M. domestica* in all traps.	Number of marked *C. macellaria* caught.	Percentage of *C. macellaria* in each trap compared with total *C. macellaria* in all traps.	Percentage of marked *C. macellaria* in each trap compared with total marked *C. macellaria* in all traps.
	Gm.	*Gm.*		*Gm.*							
1 west	368	109	29.6	259	70.4	3.29	3	4.35	48	7.84	17.64
2 west	674	262	38.9	412	61.1	7.90	1	1.45	21	12.47	7.72
3 west	242	181	74.8	61	25.2	5.46	2	2.90	4	1.84	1.47
4 west	752	596	79.2	156	20.8	17.97	3	4.35	8	4.72	2.94
1 north	316	179	56.6	137	43.4	5.40	15	21.74	38	4.15	13.97
2 north	283	238	84.1	45	15.9	7.18	13	18.84	7	1.36	2.57
3 north	361	333	92.2	28	7.8	10.04	5	7.24	0	.85	0.00
4 north	548	120	21.9	428	78.1	3.62	0	0.00	28	12.95	10.28
1 east	397	104	26.2	293	73.8	3.14	2	2.90	31	8.87	11.40
2 east	174	57	32.8	117	67.2	1.72	0	0.00	13	3.54	4.78
3 east	583	446	76.5	137	23.5	13.45	4	5.80	6	4.14	2.21
4 east	677	72	10.6	605	89.4	2.17	2	2.90	5	18.32	1.84
1 south	202	86	42.6	116	57.4	2.59	10	14.49	38	3.51	13.97
2 south	520	291	56.0	229	44.0	8.77	9	13.04	13	6.93	4.78
3 south	196	121	61.7	75	38.3	3.65	0	0.00	3	2.27	1.10
4 south	327	121	37.0	206	63.0	3.65	0	0.00	9	6.24	3.31
Total	6,620	3,316	.....	3,304	.....	100.00	69	100.00	272	100.00	99.98

FOURTH DISPERSION TEST AT DALLAS, TEX.

Since marked specimens of both the house fly and the screw-worm fly were recovered to the maximum distance afforded by the recovery traps in the third dispersion test, it was decided to perform another experiment with the recovery stations at still greater distances from the point of liberation. The same point for liberating the flies was used in this test, but owing to difficulties encountered in collecting the flies and rebaiting the recovery traps to the north it was deemed advisable to set only two traps in that direction. The number of traps to the south was also decreased to two; the number east and west was increased to five in each direction, and one trap was placed in the southeast quadrant and one in the northeast quadrant in order to show more clearly whether or not the flies were following the main arteries of traffic which run approximately north and south and east and west.

The location of the traps in this test is indicated in figure 2. Their immediate environment and distance from the point of liberation were as follows: West, No. 1, 9.5 miles, in the edge of the town of Farmers Branch, some poultry and a cow on adjacent lot; No. 2, 10.9 miles, near farmhouse, hogpen, mule and cow lot adjacent; No. 3, 13.2 miles, under tool shed at a ranch headquarters, considerable live stock kept near by; No. 4, 15.8 miles, under sheep shed on farm, horses, mules, and hogs near; No. 5, 17.8 miles, against chicken house on farm, some hogs and mules 100 yards away. North, No. 1, 13.14 miles, in edge of town of Allen, in yard near hogpen; No. 2, 16.85 miles, in old barn on farm, hogs and mules in barnyard. East, No. 1, 9.3 miles, on farm, hogs, cows, and horses in barnyard; No. 2, 10.8 miles, under shed on farm, many pigs, sheep, and other animals in yard and small slaughterhouse 100 yards distant; No. 3, 13.1 miles, under water tank on farm, all classes of stock in adjacent yard; No. 4, 15.1 miles, under shed on farm, mules, hogs, and cattle near; No. 5, 17 miles, in shed adjacent to hogpen on farm, ½ mile beyond town of Rockwall. South, No. 1, 8.2 miles, under tree in edge of Dallas, no live stock near (same situation as trap No. 4 in previous test); No. 2, 9.7 miles, at laboratory in middle of residential section of Dallas, live stock kept in yard. Southeast, so designated, one trap 7 miles from point of liberation being 2.5 miles east of south, ½ mile northwest of town of Reinhardt, under shed by barn, live stock in yard. Southwest, so designated, one trap 10 miles from point of liberation, 4 miles south of west from that point, placed by empty barn with house, hogpen, and horse lot near by.

The traps to the west, north, east, and south were located on the same roads as in the previous test. The southeast trap was near a main highway running northeast from Dallas to Garland, where it forms a juncture with a macadamized road running east from the point of liberation. Very little if any traffic would pass directly by the point of liberation and around by this trap.

The southwest trap was located on the main highway running northwest from Dallas. The traffic on this road was of about the same character and amount as on the road from Dallas north by the point of liberation. There was practically no chance of any vehicles going from the point of liberation by this trap and no roads giving direct communication between the two points.

On October 10 the first liberation was made. On the preceding day the traps were all placed and baited with fresh slime, as in the preceding experiments. About 25,000 flies colored with unfading red (paint pigment) were released at 11 a. m. These were secured by means of traps set at a packing house on the previous evening. At the time of liberation the sun was partly clouded and a slight breeze was blowing from the south. Many of the flies went several feet into the air and flew in all directions, while others settled on the hedge and vegetation near by. At 4.30 p. m. the same afternoon about 20,000 flies taken in traps at the packing house since the morning were colored in the same way and released. At that hour it was cloudy and cool. The flies did not show a tendency to remain on the cages and on the persons of the writers as in the previous experiment. Most of them settled on the hedge and other vegetation near.

On October 11, at 11 a. m., a third liberation of about 15,000 flies was made. These flies were secured the previous afternoon at the packing house. At 4.30 p. m. an additional lot of about 20,000 flies colored in the same way was liberated. The clouds were heavy and a light shower fell just prior to liberation.

A sample of the flies liberated on the first day was examined to determine the proportion of the species and sex. The results were as follows: *Musca domestica* 66.4 per cent, *Chrysomya macellaria* 28.5 per cent, *Phormia regina* 3 per cent, *Lucilia sericata* 1 per cent, *Ophyra* spp. 0.7 per cent, *Hydrotaea dentipes* Fab. 0.4 per cent. The estimated percentage of species in the lots liberated on the succeeding day agreed closely with these figures.

The approximate averages of these species in the total catch in the 16 recovery traps were: *Musca domestica* 68 per cent, *Chrysomya macellaria* 13 per cent, *Phormia regina* 10 per cent, *Ophyra* spp. 1 per cent, and other species 8 per cent.

Of the 39 marked flies recovered 33.3 per cent were *Musca domestica*, 23.1 per cent *Chrysomya macellaria*, 41 per cent *Phormia regina*, 2.6 per cent *Ophyra leucostoma*. The large percentage of *P. regina* recovered, as compared with the other species, is striking. This may no doubt be explained in part at least by the fact that the cooler temperatures of fall were more favorable for the diffusion and recovery of that species than of the house fly and screw-worm fly. In this experiment, as in the last, the percentage of marked house flies recovered is about one-half of the

percentage of this species liberated and also about one-half of the percentage of this species in the total collection in the recovery traps.

It should be stated that the weather conditions encountered in this test were somewhat adverse, especially as regards variable winds (Table XI). Furthermore, the lateness of the season probably had a markedly adverse influence on the spread of some of the species, notably *Chrysomya macellaria*, which is preeminently a warm-weather species. The test was carried out at a time when the activity and number of *C. macellaria* were declining and the reverse was true in the case of *Phormia regina*, which predominates as a fall and winter species. This is clearly indicated by the diminution in the percentage of these two species of flies taken in the various traps. During the early part of the period the total captures in certain traps averaged about 20 to 40 per cent *C. macellaria* and 7 to 10 per cent *P. regina*, while toward the end of the period the percentage of *C. macellaria* dropped to 5 or 10 and *P. regina* increased to 20 or 30. The total numbers of house flies captured were fairly large throughout the period.

Owing to the distance which it was necessary to traverse in emptying the recovery traps and rebaiting them it was deemed best to make the rounds about every three days, hence for the most part the traps were emptied, rebaited, and the flies examined on October 12, 15, 18, 21, and 29, and others were examined on November 4 and 14. The only notable exception to this arrangement was trap No. 2 south, which was emptied daily after October 15. The bait in this trap was thus given somewhat better attention, and this gave it a slight advantage over the other traps.

DISTANCE OF DISPERSION

The distance the different species of marked flies had traveled is shown in Table X. It will be noted that in general in the nearer traps ranging from 7 to 11 miles the major part of the marked flies were captured. In the more distant traps ranging from 13 to 15 miles only two marked flies were recovered, while in the traps approximately 17 to 18 miles from the point of liberation no liberated flies were taken. The maximum distances recorded for the four species of flies recovered were: *Musca domestica* 13.14 miles, *Chrysomya macellaria* 15.1 miles, *Phormia regina* 10.9 miles, *Ophyra leucostoma* 7 miles.

TABLE X.—*Fourth dispersion test at Dallas, Tex. Species and sex of marked flies recovered in rings of traps about point of liberation*

Trap No.	Distance from place of release.	*Musca domestica.*		*Chrysomya macellaria.*		*Phormia regina.*		*Ophyra leucostoma.*		Total.		Grand total.
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
S. E.	7.0	0	6	0	0	0	2	1	0	1	8	9
1 S.	8.2	0	1	0	1	0	1	0	0	0	3	3
1 W.	9.5	0	0	0	2	0	2	0	0	0	4	4
1 E.	9.3	0	1	0	0	0	0	0	0	0	1	1
2 S.	9.7	0	3	0	1	1	3	0	0	1	7	8
S W.	10.0	0	0	0	2	0	3	0	0	0	5	5
Total		0	4	0	5	1	8	0	0	1	17	18
2 W.	10.9	0	1	0	2	0	3	0	0	0	6	6
2 E.	10.8	0	0	0	0	0	1	0	0	0	1	1
Total		0	1	0	2	0	4	0	0	0	7	7
1 N.	13.14	0	1	0	0	0	0	0	0	0	1	1
3 W.	13.2	0	0	0	0	0	0	0	0	0	0	0
3 E.	13.1	0	0	0	0	0	0	0	0	0	0	0
Total		0	1	0	0	0	0	0	0	0	1	1
4 W.	15.8	0	0	0	0	0	0	0	0	0	0	0
4 E.	15.1	0	0	0	1	0	0	0	0	0	1	1
Total		0	0	0	1	0	0	0	0	0	1	1
5 W.	17.8	0	0	0	0	0	0	0	0	0	0	0
3 N.	16.85	0	0	0	0	0	0	0	0	0	0	0
5 E.	17.0	0	0	0	0	0	0	0	0	0	0	0
Total		0	0	0	0	0	0	0	0	0	0	0
Grand total		0	13	0	9	1	15	1	0	2	37	39

TABLE XI.—*Climatological data relating to fourth dispersion test at Dallas, Tex.*

Date.	Temperature.			Wind.		Rain.	Humidity.			Actual sum.
	Max.	Min.	Mean.	Direction during successive hours from 6 a. m. to 8 p. m.	Velocity during day.		Max.	Min.	Mean.	
1918.	°F.	°F.	°F.		Miles.	Inch.	Per cent.	Per cent.	Per cent.	Per cent.
Oct. 10	76	64	70.0	4 SE., 3 S., 7 NE.	3 to 5	0	90	66	78	8
11	77	64	70.5	3 SE., 5 S., 3 W., 3 N.	2 to 7	0.02	99	72	85	7
12	84	62	73.0	6 E., 2 S., 2 NE., 4 N.	1 to 8	0	100	57	78	70
13	83	64	73.5	2 N., 3 NW., 5 NE., 4 W.	1 to 10	Trace.	87	60	73	67
14	79	59	69.0	3 NE., 3 E., 8 NE.	5 to 16	0	50	25	37	100
15	76	49	62.5	3 NW., 11 NE.	2 to 10	0	74	35	54	94
16	76	62	69.0	2 N., 5 NE., 3 N., 4 NE.	6 to 10	0	66	50	58	35
17	72	63	67.5	4 E., 3 NE., 5 N., 2 NE.	3 to 7	.18	97	84	90	4
18	79	66	72.5	5 N., 2 NE., 2 W., 5 NW.	3 to 7	Trace.	94	66	80	45
19	79	64	71.5	2 NW., 7 W., 2 NW., 3 N.	2 to 7	0	94	78	86	35
20	86	68	77.0	3 NW., 4 N., 2 NW., 3 N.	3 to 9	Trace.	83	50	66	62
21	81	64	72.5	4 E., 4 SE., 3 E., 3 NW.	5 to 10	0	87	64	75	45
22	69	63	66.0	5 SE., 4 S., 2 SE., 3 E.	2 to 17	2.76	100	94	97	0
23	69	62	65.5	1 N., 2 NW., 4 N., 7 NW.	3 to 12	Trace.	97	80	88	18
24	69	62	65.5	11 NW., 3 W.	3 to 11	0	87	66	76	14
25	74	56	65.0	2 E., 11 SE., 1 E.	2 to 13	0	97	60	78	79
26	66	43	54.5	4 SE., 3 N., 7 W.	8 to 20	2.66	99	91	95	0
27	59	42	50.5	12 W., 2 SW.	3 to 18	0	79	56	67	65
28	71	46	58.5	1 SE., 11 S., 2 SE.	8 to 32	0	59	28	43	100
29	67	54	60.5	1 W., 13 NW.	7 to 13	0	89	47	68	55
30	68	47	57.5	10 NW., 4 W.	3 to 10	0	95	47	71	100
31	64	48	56.0	4 W., 6 NW., 4 N.	4 to 10	0	80	37	58	100
Nov. 1	69	42	55.5	10 SE., 1 S., 3 SE.	5 to 13	0	76	29	52	100
2	73	48	60.5	14 SE.	7 to 18	0	72	34	53	94
3	75	52	63.5	4 SE., 6 S., 4 SE.	6 to 12	0	94	60	77	68
4	75	54	64.5	3 SE., 6 S., 5 SE.	5 to 12	0	99	60	79	62
5	76	57	66.5	14 SE.	7 to 18	Trace.	94	75	84	22

DIRECTION OF DISPERSION

The variation in the distances at which the traps in different directions from the point of liberation were located makes it somewhat more difficult to determine the tendencies of the marked flies to travel in different directions. Although the southeast trap was nearest to the point of liberation, it is notable that in it six flies, nearly 50 per cent of the marked house flies, were recovered, the next largest number (4) being in the two traps to the south, one in the east, one in the north, one in the west, and none in the southwest, thus showing with this species a tendency to travel toward the south and southeast. On the other hand, the tendencies as regards direction of flight of *Chrysomya macellaria* and *Phormia regina* appear to differ considerably from those of *Musca domestica*, but are themselves similar. Four (44.4 per cent) *C. macellaria* and five (31.25 per cent) *P. regina* were taken in the west traps, two (22.2 per cent) *C. macellaria* and three (18.75 per cent) *P. regina* in the southwest traps, two (22.2 per cent) *C. macellaria* and five (31.25 per cent) *P. regina* in the south traps, one each (11.1 and 6.25 per cent) in the east traps, and two (12.5 per cent) *P. regina* in the southeast, thus showing in these species a tendency toward migration to the west, southwest, and to a lesser degree to the south.

As is shown in Table XI, the wind during this entire period of recovery was very choppy. During the first 2 days after release there was some south and southeast wind, but aside from this there was during the recovery period a very marked predominance of north and northeast wind. This would seem in the case of the two blowflies to indicate a tendency to travel with the wind in this test. Up to the end of October 16, when recoveries of marked blowflies had nearly ceased, there was experienced during the daylight hours 68 hours of wind from the north, northeast, and east, compared with 17 hours from the west, southwest, and south. The drawing of conclusions on this point, however, would seem unwarranted owing to the extreme variability of the wind during the test.

A considerable number of house flies taken in the southeast trap and of blowflies taken in this and the southwest trap indicates still further that dissemination by following vehicles along the roads is probably not an important factor in this or the previous tests.

In this test, as in the previous one, the variable wind made it difficult to determine whether attractive odors such as those produced by towns and cities are an important influence in determining direction or distance of flight.

RELATION OF SEX TO DISPERSION

In this test all of the 13 marked house flies recovered were females, as were the 9 screw-worm flies and all but 1 (6.25 per cent) of the 16 specimens of *Phormia regina*. The only specimen of *Ophyra leucostoma* taken was a male.

The proportion of sexes among the released flies as indicated by examination of a sample at the time of release was: *Musca domestica*, 34 per cent males, 66 per cent females; *Chrysomya macellaria*, 6 per cent males, 94 per cent females; *Phormia regina*, 22 per cent males, 78 per cent females; *Ophyra leucostoma*, 100 per cent males; *Lucilia sericata*, 100 per cent females. In the case of the house fly we would have expected to recover a few males if that sex had shown migratory tendencies equal to the female, but with *C. macellaria* the percentage of males released was so small and the number of marked flies recovered so few that we would hardly expect to recapture any males.

RELATION BETWEEN POSITION OF TRAP AND NUMBER AND SPECIES OF FLIES CAPTURED

As in the previous test, the number and percentage of different species of flies captured in the different traps varied considerably. During the period covered by the test a total of 12,811 gm. of flies were taken. In this test the average catch per trap of all species was: West, 762 gm.; north, 1,212 gm.; east, 587 gm.; south, 1,229 gm.; southeast, 454 gm.; southwest, 732 gm. The size of the total catches in different traps in either direction varied greatly, especially in the five traps to the west. It appears quite certain that traps set in situations favorable for flies and where a comparatively large catch is obtained would be more likely to capture marked individuals coming to that locality. Furthermore, those traps which are situated most favorably for catching house flies would, other conditions being equal, show a larger percentage of marked house flies as compared with marked blowflies, and the reverse is equally true. This factor probably had some influence on the results in the present test, although the comparatively small number of marked flies captured makes the drawing of conclusions on this point unwarranted.

LONGEVITY OF MARKED FLIES

In addition to the fact that we are concerned with the possible adverse effects of handling and marking the flies used in these various tests, there is presented an opportunity of determining the facts as to the longevity of several species of flies under natural or unrestrained conditions.

It is apparent also that longevity must play an important part in the securing of records on dissemination by the method used in these tests. Since few flies were colored in proportion to the area covered by the recovery traps, especially in the last two tests, and since under such conditions we can hope to capture but a small portion of the flies in the area or even in the vicinity of the traps, each additional day of life and activity of the marked flies enhances the chances of capturing them.

In the several tests the following records of longevity were obtained: First test at Dallas (radius of dispersion 3 miles), *Musca domestica* 11 to

12 days, *Chrysomya macellaria* 7 to 8 days, *Phormia regina* 5 to 6 days, *Lucilia sericata* 5 to 6 days, *Sarcophaga* spp. 11 to 12 days, *Ophyra leucostoma* 3 to 4 days. Second test at Dallas (radius of dispersion 5 miles), *M. domestica* 9 to 11 days, *C. macellaria* 9 to 11 days, *P. regina* 8 to 10 days, *Sarcophaga* spp. 2 to 4 days, *O. aenescens* 6 to 8 days. Third test at Dallas (radius of dispersion 8 miles), *M. domestica* 15 days, *C. macellaria* 17 days, *O. aenescens* 4 days. Fourth test at Dallas (radius of dispersion 17 miles), *M. domestica* 8 to 9 days, *C. macellaria* 10 to 11 days, *P. regina* 10 to 11 days, *O. leucostoma* 1 to 5 days.

In considering the radius of dispersion it should be borne in mind that the marked flies may not necessarily have remained constantly within the circle with the radius given but may have returned into it after more extended travel.

STIMULI AFFECTING DISPERSION

The evidence gained points toward the operation of several stimuli in initiating and governing dispersion. Under the conditions obtaining in nature these stimuli seem to be so blended or mixed as to make it impossible to weigh their relative importance or, in fact, to determine the nature of some of them. It seems probable that the strength of these stimuli and even their character vary with different species.

The importance of food and favorable breeding places as stimuli is clearly shown by the recovery of the greater numbers of specimens of a species in traps located near favorable feeding and breeding grounds. Behind this immediate desire of the individual to obtain food and find a suitable breeding place there appears to be an inherent tendency to disperse. There seems to be some evidence that the house fly was influenced in regard to direction of dispersion by the proximity of farm buildings and towns, indicating spread from one attractive point to another, rather than long sustained flight.

The recovery of males as well as females far from the point of liberation indicates strong dispersion tendencies in that sex as well as in the females. Since mating does not usually take place immediately after emergence, it is no doubt important for the males to seek breeding places where the females are to be found, and they are also attracted to food though not so strongly as are the females.

The question has often been asked, do the flies when liberated tend to return to their original habitat; in other words, is the homing instinct developed? These tests were not planned to determine this point, though they happen to give data on it. The figures presented under the discussion of direction of flight clearly show that there is no marked tendency for any of the species to travel back toward their original place of capture. In this connection it should be remembered that in the first and second tests at Dallas the west traps were nearly in line

from the place of capture to the point of release, and in the third and fourth tests the south traps were about in line.

Climatic influences are unquestionably important in relation to the spread of these species of Diptera. Probably temperature, humidity, sunshine, wind (direction and velocity), precipitation, barometric pressure, and electrical phenomena each exert an influence. It is difficult in experiments of this kind to weigh reliably these various factors because the results are extended over a considerable period with a corresponding change in meteorological conditions, and hence an equalizing of the effects of the stimuli. Those factors which would stimulate activity, such as high temperatures and sunshine, are no doubt potent. Another stimulus is probably to be found in those combinations of climatic conditions experienced with the change of seasons. This seasonal stimulus may account for the marked activity in dispersion and food-seeking found in *P. regina* in the fourth experiment.

COMPARATIVE TENDENCIES OF THE SPECIES TO DISPERSE

The relative tendencies of the several species toward dispersion are suggested by a comparison of the percentages of marked specimens recovered with those liberated and those in the total catch in the recovery traps. These figures indicate that this tendency is more marked in *Chrysomya macellaria* than in *Musca domestica*. The percentage of marked *C. macellaria* as compared with the total marked flies recovered in all of the distant migration tests nearly equaled or surpassed the percentage of that species among the flies liberated, while in *M. domestica* the percentage of marked specimens recovered was approximately one-half of the percentage liberated. Under favorable climatic conditions for the species, *Phormia regina* showed a very strong migratory tendency, the percentage of marked specimens recovered in the third test being 14 times the percentage of that species in the release. *Ophyra leucostoma* and *O. aenescens* also exhibit a decided tendency toward dissemination, the percentage of marked specimens of these species recovered being in all cases greater than in the liberation. The number of specimens of other species liberated was too few to be used in this comparison, but several appear to travel quite freely.

SUMMARY AND CONCLUSIONS

The dispersion of several species of flies important in the economy of man, both as carriers of disease and as parasites of man and animals, is discussed in this paper.

The experiments carried out show that under rural and urban conditions flies have marked powers of diffusion.

The maximum distance of spread from the point of release as recorded in these tests was as follows for the several species: *Musca domestica*,

13.14 miles; *Chrysomya macellaria*, 15.1 miles; *Phormia regina*, 10.9 miles; *Lucilia sericata*, 1.2 miles; *L. caesar*, 3.5 miles; *Synthesiomyia brasiliana*, 0.5 mile; *Sarcophaga* spp., 3 miles; *Ophyra leucostoma*, 7 miles; *O. aenescens*, 4.1 miles.

The estimated total number of marked flies liberated in all the experiments reported upon was 234,000.

In these tests it is considered that too few individuals of species other than *Musca domestica*, *Chrysomya macellaria*, and *Phormia regina* were liberated to form a reliable guide to their dissemination tendencies.

Marked flies of all species dispersed in all directions from the point of liberation.

Among the stimuli inducing dispersion the desire for food and the desire for places for oviposition appear to be among the strongest.

The fact that many towns, farmhouses, and other favorable feeding and breeding grounds were passed by the flies shows that *Musca domestica*, *Chrysomya macellaria*, and *Phormia regina* at least are not satisfied by the mere finding of these places but have marked migratory habits.

Chrysomya macellaria evinces stronger tendencies toward migration than does *Musca domestica*. This tendency in *Phormia regina* under optimum climatic conditions for the species is probably equal to that in *C. macellaria*. The other species were liberated in numbers too few for conclusions to be drawn, but *Ophyra leucostoma* and *O. aenescens* show marked ability to travel considerable distances.

The exact relation between direction of dispersion and direction of wind could not be determined from the results of these experiments because of the choppy wind conditions experienced. There appears to be a tendency for *Musca domestica* and *Chrysomya macellaria* to go with the wind in greatest numbers, but they are shown to travel against and at right angles with it as well. It is concluded that under natural conditions the influence of moderate winds on dissemination is not of great importance.

The evidence gained justifies the conclusion that the passing of vehicles along the highways was not a dominating factor in the dispersion of any species of flies in these tests. This does not mean, however, that flies under other conditions may not be widely scattered by artificial means.

These tests show that the house fly, screw-worm fly, and black blowfly spread rapidly for many miles. *Chrysomya macellaria* was recorded about 8 miles from the point of liberation in less than 24 hours and 10 miles in less than 48 hours after liberation. *Phormia regina* was recovered about 11 miles away in less than 48 hours after release. *Musca domestica* was recovered over 6 miles from the point of release in less than 24 hours.

Males as well as females of the principal species used in these experiments may travel many miles.

The maximum longevity of the marked flies after liberation as shown by the records of capture was: *Musca domestica* 15 days, *Chrysomya macellaria* 17 days, *Phormia regina* 10 to 11 days, *Ophyra aenescens* 6 to 8 days, *Sarcophaga* spp. 11 to 12 days.

While in the fourth experiment no marked flies were captured in the more distant traps (about 17 miles from the point of release), it is the authors' belief that the limits of dispersion were not reached in that test and that where great numbers of flies are emerging constantly the distance traversed may be much farther than the maximum here determined.

The facility with which flies travel many miles emphasizes the importance of the general application of sanitary measures looking toward the suppression of fly breeding.

BACTERIOLOGICAL AND CHEMICAL STUDIES OF DIFFERENT KINDS OF SILAGE

By CHARLES A. HUNTER,[1] *Bacteriologist, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

The aim of this investigation was to study the nature of the fermentations taking place in silage composed of a mixture of silage crops. The investigation was occasioned by the fact that there seems to have been considerable disagreement in the conclusions drawn by different investigators. Through a review of the literature it was noted that plant enzyms were first thought to be the important factors. Recently it has been shown that microorganisms appear to play an important part. Those who favor this latter conclusion claim that the production and increase in acidity are caused by bacterial action. Still more recently some investigators have stated that fermentations were due to the combined action of plant enzyms and microorganisms and that the former of these was the more important factor.

It is definitely known that as silage fermentations take place there is an increase in the volatile and nonvolatile acids and ammonia nitrogen. It has been claimed that the hydrolsis of protein is first caused by enzyms and later by microorganisms.

Corn, kafir corn, cane, corn stover, alfalfa, and alfalfa with a carbohydrate supplement have all been used to make the silage upon which different investigators have worked. In a search of Experiment Station literature the author was unable to find that any bacteriological and chemical studies had been made on silage composed of a mixture of silage crops. It was therefore deemed desirable to study two of the most common silage mixtures now used on Pennsylvania farms—Canada field peas with oats and corn with soybeans. The nature of fermentations in silage made from these two mixtures was studied in the seasons of 1918 and 1919.

REVIEW OF LITERATURE

Babcock and Russell (*1*, *2*)[2] found that silage would undergo fermentation even when treated with chloroform, ether, and benzene which inhibited the growth of microorganisms. They found also that the amount of heat and acids increased and concluded that this was the result of intramolecular respiration. E. J. Russell (*16*) came to nearly the same conclusion as Babcock and Russell in deciding that plant cells and

[1] Special credit is due Prof. S. I. Bechdel, of the Department of Dairy Husbandry, who stored the crops in the large silos, assisted very materially in the securing of the representative samples as they were needed from time to time, helped make the determinations of the total and volatile acids, and gave invaluable assistance in preparing the manuscript.

[2] Reference is made by number (italic) to "Literature cited," p. 788–789.

enzyms were the primary and essential factors in silage fermentation and that bacteria played a minor part.

Esten and Mason (*6*) attributed the fermentation largely to the work of bacteria and yeasts. They concluded that there were three chief fermentations, lactic acid, alcohol, and acetic acid. The primary change was the fermentation of sugar by organisms similar to the common milk-souring organisms which converted it to lactic acid. The secondary change was produced by yeast which converted the remaining sugar to alcohol, the acetic acid bacteria then oxidizing the alcohol to acetic acid.

Samarani (*17*) believed that the sugars were transformed to alcohol and later oxidized to acetic acid by respiration of plant cells. The formation of lactic acid was the result of the action of a bacillus and a coccus which he found in almost equal proportions. He thought the organisms were the same as those that are common in milk fermentation.

Hunter and Bushnell (*13*) conclude that microorganisms cause the major fermentation of silage. They found large numbers of organisms belonging to the bulgaricus group present and state that these organisms are responsible for the high acid content of silage. Organisms belonging to the bulgaricus group and the colon group produce the greater part of acetic acid. Sherman (*18*) has also shown that silage contains large numbers of the organisms belonging to the bulgaricus group.

Lamb (*14*) states that—

> neither microorganisms nor plant enzymes are alone responsible for the changes in silage fermentation.

Microorganisms are largely responsible for the production of acid and the disappearance of sugars. He also states that the formation of alcohols and the hydrolysis of protein as indicated by the amino-nitrogen content are primarily due to cell respiration, although later in the fermentation microorganisms show some activity in both processes.

Hunter (*11*) has shown that the heat production in forage silage is due to microbial activity and not to intramolecular respiration of plant tissue. In his studies on alfalfa silage (*12*) he found that alfalfa alone made a silage of inferior quality but that upon the addition of an available carbohydrate supplement a good quality of silage could be produced. The protein hydrolysis of alfalfa silage as indicated by the amino-nitrogen and ammonia determinations was greater than when a carbohydrate was present. Round (*15*), working on the fermentation of sauerkraut, found that cell respiration plays a more important part than was formerly thought.

Sherman and Bechdel (*19*), working on the fermentation of corn stover silage, found that it undergoes fermentation similar to corn silage. The following statement is also made:

> From a review of the present status of the question as to whether bacteria or plant cells are mainly responsible for silage fermentation, it is concluded that the data thus far published are inconclusive. Although the results of the present study tend to support the cell respiration theory, conclusions on this point are withheld.

METHOD OF PROCEDURE

Two main sets of experiments were planned—first, field experiments; second, laboratory experiments. Several investigations were planned under each set. It was planned to make an analysis at the time of storage, every other day for a week, every three days for the next week, and then once every week until the silage was about a month old. There were times in making these analyses when it was necessary to deviate slightly from the original plans.

SILOS

The two silos used in storing the different kinds of field silage were of the monolithic concrete type with inside dimensions of 8 feet in diameter and 30 feet in height. The walls, 6 inches in thickness, were nonporous and the inside was made smooth with a coat of plaster. To afford a means of sampling, pieces of 2-inch water pipe 6 inches long were placed in the wall at the time the silos were constructed. These sample holes were located 3 to 5 feet apart all the way around the silos to a height of 12 feet and were plugged.

The samples used in all tests were obtained by making repeated borings through these holes with a 2-inch auger. A new hole was used for obtaining material for each successive analysis. The auger was provided with an extension shaft 8 feet in length so as to penetrate to the center of the silo.

The silos used for laboratory experiments consisted of quart milk bottles, stoppered with rubber stoppers wired in, and sealed with paraffin. One bottle of silage was used for each day's analysis.

BACTERIOLOGICAL METHODS

Samples of silage were collected from the concrete silos in sterile containers and taken to the laboratory. In order to secure a representative sample the silage was passed through a sterile meat grinder. Twenty gm. of this silage were placed in 200 cc. of sterile physiological salt solution. This was thoroughly shaken, and further dilutions were made.

TOTAL NUMBER OF MICROORGANISMS.—In determining the total number of bacteria, plain agar was used. No doubt a somewhat greater number of organisms could have been obtained with a carbohydrate medium, but the relative increase or decrease in the number would have been the same.

TOTAL ACID PRODUCERS.—Dextrose litmus broth and dextrose cresol purple broth were used. Dextrose litmus broth was prepared by using 1 per cent dextrose broth to which litmus had been added. Cresol purple solution was made by dissolving 0.4 gm. of di-bromo-ortho-cresol-sulphonphthalein in a minimum amount of alcohol and making up to 1 liter with water. Forty cc. of this were added to 1 liter of dextrose broth. The broths were inoculated with different dilutions of the silage

infusion and incubated. Acid formation in the cresol purple broth is indicated by a yellow color. This color was very distinct, and only a trace of acid present caused the broth to change from purple to yellow. In litmus the results were at times rather indistinct; moreover, the litmus was not as sensitive to small amounts of acid as cresol purple. Cresol purple was used 50 times in comparison with litmus. Cresol purple gave more organisms 14 times; litmus gave higher results 9 times; and equal results were obtained 27 times. Expressed in percentage, the greater number of bacteria were found in the cresol purple solution in 28 per cent of the times, in litmus 18 per cent, and in equal numbers 54 per cent.

COLON-AEROGENES GROUP.—Bile lactose broth and lactose broth were used to determine the number of colon organisms present. Dunham fermentation tubes containing approximately 10 cc. of respective media were inoculated with 1 cc. of various dilutions of the silage infusions. The number of organisms present was determined from the tube showing gas in the highest dilution. In order to verify the presumptive test, colon organisms were isolated at different intervals. Bile lactose broth and lactose broth were used 55 times in comparison with each other. Lactose broth gave more organisms 8 times, bile lactose broth gave more 11 times, and equal results were obtained 36 times. In terms of percentage, lactose broth gave the greater number of organisms in 14.6 per cent of the determinations, bile lactose in 20 per cent, and equal results were obtained in 65.4 per cent.

BULGARICUS.—The plate method was used to determine the number of bulgaricus organisms present. One per cent dextrose agar was used to which 1 cc. of a 1 per cent solution of sterile acetic acid was added at the time the plates were poured. From time to time organisms belonging to the bulgaricus group were isolated and identified.

YEAST.—The dilution method was used to determine the number of yeasts present. One per cent dextrose broth in Dunham fermentation tubes was inoculated with 1 cc. of the dilutions of silage extract. The formation of gas was taken as an indication that yeasts were present. This, however, was confirmed by microscopic examination.

PROTEIN DIGESTERS.—Gelatin was inoculated with 1 cc. of the various dilutions and incubated at 37° C. The gelatin upon being removed from the incubator was placed in a refrigerator. Gelatin which has been digested will not solidify upon cooling; hence by taking the highest dilution showing liquefaction the number of protein digesters could be determined.

All media used were made according to standard methods and were titrated with brom-thymol blue (di-brom-thymol-sulphonphthalein). This gave the media a hydrogen concentration of 6.4–7.6 (*4*). The period of incubation was seven days at 37° C. Direct microscopic counts of the silage juice were made with Breed's method. The results obtained compared very favorably with those secured by the plate method.

CHEMICAL METHODS

From the sample of silage the juice was expressed and used for the chemical analysis.

TOTAL ACIDITY.—Ten cc. of juice were diluted with 990 cc. of distilled water and titrated with *N/10* barium-hydroxid solution, using phenolphthalein as an indicator.

VOLATILE ACIDITY.—Twenty-five cc. of juice were subjected to steam distillation under reduced pressure. One liter of distillate was secured and titrated immediately with *N/10* barium hydroxid, using phenolphthalein as an indicator. Experiences in other investigations have established the efficiency of this method (*5*).

AMINO NITROGEN.—The amino nitrogen was determined by the use of the Van Slyke apparatus (*20*).

AMMONIA NITROGEN.—The ammonia was determined by the use of Folin's method, using 25 cc. of juice and aerating for at least eight hours.

ALBUMINOID NITROGEN.[1]—This was run on the first and last sample of most of the silage. It was determined by Stutzer's method.

TOTAL NITROGEN.[1]—The total nitrogen was determined by the Kjeldahl method.

MOISTURE.—One thousand gm. of a well-mixed sample of the silage were placed in an air-drying oven for at least three days.

FIELD EXPERIMENTS

Three separate field experiments were performed:

I. Canada field peas and oats, 1918.
II. Canada field peas and oats, 1919.
III. Corn in comparison with corn and soybean mixture, 1918.

EXPERIMENT I. CANADA FIELD PEA AND OAT SILAGE, 1918

The crop of oats and peas was seeded May 6 and stored in the silo July 17. The oats were in the milk stage of growth and the peas were well formed in the pod. The fresh green mixture at the time of ensiling contained, on the average, 18.4 per cent peas. On account of the extremely dry weather for the two weeks just previous to harvesting, the moisture content was rather low, being only 70 per cent. The silage mixture kept very well and was eaten with relish by the cows on the experiment. Feeding was not begun until August 8 so as not to interfere with the method of securing samples. The results of the bacteriological examination are given in Table I.

[1] The analytical work of determining the total and albuminoid nitrogen was done by Mr. Walter Thomas, Assistant Chemist of the Pennsylvania Agricultural Experiment Station.

TABLE I.—*Results of bacteriological examination of Canada field pea and oat silage in concrete silo*

[Expressed in number of bacteria per gram]

Date sampled.	Number of days from ensiling.	Total in plain agar.	Total acid producers.		Colon group.		Bulgaricus group.	Yeast.	Liquefiers in gelatin.
			Dextrose litmus broth.	Cresol purple broth.	Bile lactose broth.	Lactose broth.			
1918.									
July 19	0	1,800,000	100,000,000	1,000,000,000	1,000,000	10,000,000	600	10,000,000	1,000
20	1	550,000,000	100,000,000	100,000,000	100,000,000	100,000,000	265,000	100,000	10,000,000
23	4	150,000,000	100,000,000	10,000,000	710,000,000	710,000,000	25,000,000	100,000	100,000
25	6	97,000,000	1,000,000	1,000,000	10,000	10,000	735,000	100,000	100,000
27	8	280,000,000	100,000,000	100,000,000	710,000,000	10,000,000	.....	10,000	100,000,000
30	11	.....	1,000,000	10,000,000	0	0	18,500	1,000,000	1,000,000
Aug. 3	15	.....	10,000,000	1,000,000	100,000	100,000	.....	100,000	1,000,000
7	19	3,500,000	10,000,000	10,000,000	10,000,000	10,000,000	543,000	10,000	10,000
14	26	6,900,000	10,000,000	10,000,000	10,000,000	10,000	31,000	100,000	10,000

From the results in Table I it can be noted that the oats and Canada field peas at the time of ensiling contained a large number of organisms. The acid-producing organisms were predominating. Organisms belonging to the bulgaricus group were present in very small numbers. Yeasts and organisms belonging to the colon group were present in large numbers. There were few protein digesters present at the time of ensiling. As fermentation progressed the number of acid formers remained fairly constant for the first eight days and then slightly decreased. The bulgaricus group increased rapidly for the first four days; then there was a gradual decrease. The number of yeast and protein digesters did not decrease as they did in some of the later experiments. One reason, no doubt, was the fact that the silage material was cut very coarse so that the silage did not compact properly, and air pockets were formed. The count upon plain agar shows an increase, then a decrease. The number of organisms belonging to the colon group increased, disappeared on the eleventh day, and then increased.

It is true that the changes which apparently took place in the silage might possibly be ascribed to variations in the samples of silage which were taken from different parts of the silo, but on account of the progressive nature of the changes taking place as shown by the increase in numbers of bacteria and in the formation of chemical compounds it would seem probable that the changes were due to the agents causing the fermentation.

EXPERIMENT II. CANADA FIELD PEA AND OAT SILAGE, 1919

The crop in 1919 was seeded earlier than in 1918 and was ready for the silo on July 3. The growing season was also very dry in 1919 until about two weeks before harvesting, and as a result the crop yield was low. The moisture content of the fresh green material was 73.8 per cent, or a little higher than that of the 1918 crop. The percentage of peas was also slightly higher, being 21.1 per cent in this investigation. A feeding test was conducted in which the silage was compared with ordinary corn silage for milk production. It proved to be very good feed, and the cows consumed it in large quantities with very good results in milk production.

Table II shows the bacteriological results obtained from this experiment. It can be noticed that the predominating group of organisms were the acid producers, of which a large percentage, no doubt, belonged to the bulgaricus group. After the first day of fermentation practically no organisms belonging to the colon group nor any yeasts were present. There were few protein digesters present.

The chemical results given in Table III show a decided increase in the total acidity, volatile acidity, and amino nitrogen during the first few days of fermentation. This was then followed by a gradual increase until the twenty-ninth day, when analyses were stopped. Ammonia determination fluctuated but showed gradual increase.

TABLE II.—*Results of bacteriological examination of Canada field pea and oat silage in concrete silo*

[Expressed in number of bacteria per gram]

Date sampled.	Number of days from ensiling.	Total in plain agar.	Total acid producers in cresol purple broth.	Colon group.		Bulgaricus group.	Yeasts.	Liquefiers in gelatin.
				Bile lactose broth.	Lactose broth.			
1919.								
July 3	0	Less than 10,000	1,000,000	1,000	1,000	Less than 100	100	1,000
7	4	1,580,000	100,000,000	0	0	250,000	10	10,000
9	6	73,000	100,000,000	0	0	1,760,000	0	10,000
11	8	.....	10,000,000	0	10	1,740,000	100	10,000
14	11	3,000,000	100,000,000	0	0	225,000	0	10,000
16	13	40,000	100,000	0	100	290,000	10	.....
18	15	350,000	100,000	0	0	275,000	0	100
25	22	.....	100,000	0	0	250,000	0	10
Aug. 1	29	2,000,000	10,000,000	0	10	4,000,000	0	7,000

TABLE III.—*Results of chemical analyses of Canada field pea and oat silage in concrete silo*

[Expressed in milligrams per 100 cc. of juice]

Date sampled.	Number of days from ensiling.	Moisture.	Total acidity in terms of lactic acid.	Volatile acidity in terms of acetic acid.	Nonvolatile acidity by difference.	Amino nitrogen.	Ammonia nitrogen.	Milligrams albuminoid nitrogen in 100 gm. dry silage.	Milligrams nitrogen in 100 gm. dry silage.
1919.		*Per cent.*							
July 3	0	73.82	984.5	29.23	955.27	176.5	54.4	95.0	157.0
7	4	62.7	3,199.30	663.8	2,535.50	271.4	54.4	.....	166.5
9	6	70.8	3,222.18	625.0	2,597.18	322.7	65.2	.....	168.0
11	8	72.9	3,288.81	604.1	2,684.71	257.6	44.8	.....	154.0
14	11	67.3	3,641.85	806.9	2,834.95	389.7	88.4	.....	183.0
16	13	70.6	3,376.32	769.66	2,579.66	314.7	61.2	.....	164.0
18	15	71.2	3,339.53	751.76	2,587.77	333.2	69.3	.....	157.0
25	22	70.9	3,377.32	818.88	2,558.44	339.1	65.2	.....	156.0
Aug. 1	29	66.7	3,759.21	1,005.3	2,753.91	416.5	76.84	79.5	180.5

It is of interest to note that the decided increase in number of acid producers and the decided increase in acidity occur at the same time, thus indicating that acidity is due to micro-organisms.

EXPERIMENT III. CORN SILAGE AND CORN AND SOYBEAN SILAGE, 1918

The same variety of corn was grown in each case. The corn-soybean mixture was obtained by planting soybeans with a hand seeder in the same rows with the corn. In proportion by green weight the soybeans in the corn-soybean mixture amounted to approximately 30 per cent. As large a percentage of soybeans as this could not be hoped for under ordinary conditions, since the viability of seed corn used was rather low, and the number of stalks was little more than half that of a normal stand.

Ensiling took place when the corn was well glazed and when the soybeans were well podded. It was necessary to add some water to the corn silage to insure its preservation, as it was somewhat advanced in maturity.

On account of the rainy season both kinds of silage contained an unusual amount of weeds. It is not believed that this affected the quality materially, since in a feeding test which followed the animals relished and ate them both throughout the experiment with practically no waste.

This experiment was run for the purpose of comparing corn silage with silage composed of a mixture of corn and soybeans. (Tables IV to VII.)

The bacteriological results given in Tables IV and VI show little difference between the two kinds of silage.

Acid-producing organisms were the predominating group. For the first few days there were large numbers of organisms belonging to the colon group, but there was a gradual reduction until on the thirty-ninth day, when there were no colon organisms in the corn silage and only a few in the corn and soybean silage. There was a gradual reduction in the number of yeasts and protein digesters in both kinds of silage.

The chemical analyses given in Tables V and VII show that the greatest increase in acidity and amino nitrogen occurs during the first four days of the fermentation. The ammonia determinations fluctuate to a considerable extent, with a tendency to decrease in the corn silage and increase in the corn and soybean silage.

Graphs showing the increase in acidity, amino nitrogen, and ammonia are given in figures 1, 2, and 3, respectively.

LABORATORY EXPERIMENTS

Three laboratory experiments were performed with Canada field pea and oat silage:

IV. Untreated.

V. Treated with 2 per cent chloroform.

VI. Sterilized and inoculated.

TABLE IV.—*Results of bacteriological examination of corn silage in concrete silo*

[Expressed in number of bacteria per gram]

Date sampled.	Number of days from ensiling.	Total in plain agar.	Total acid producers.		Colon group.		Bulgaricus group.	Yeast.	Liquefiers in gelatin.
			Dextrose litmus broth.	Cresol purple broth.	Bile lactose broth.	Lactose broth.			
1918.									
Sept. 9	0	30,000,000	10,000,000	10,000,000	10,000,000	100,000	4,400	10,000	1,000,000
10	1	23,000,000	100,000,000	100,000,000	10,000,000	1,000,000	1,670,000	1,000	100,000
12	3	880,000,000	1,000,000,000	100,000,000	1,000,000	1,000,000	417,000,000	100,000	1,000,000
13	4	590,000	1,000,000,000	1,000,000,000	100,000	100,000	162,000,000	10,000	100,000
14	5	130,000	100,000,000	1,000,000,000	100,000	100,000	14,000,000	10,000	10,000
16	7	65,000,000	1,000,000,000	100,000,000	10,000	100,000	7,000,000	.....	10,000
18	9	.....	1,000,000	100,000,000	10,000	10,000	15,200,000	10,000	.....
20	11	3,500,000	.....	100,000,000	10,000	10,000	43,000,000	.....	1,000
27	18	.....	.....	100,000,000	100	100	33,000,000	.....	10,000
Oct. 18	39	360,000	.....	1,000,000,000	0	0	17,000,000	.....	.....

TABLE V.—*Results of chemical analysis of corn silage in concrete silo*

[Expressed in milligrams per 100 cc. of juice]

Date sampled.	Number of days from ensiling.	Moisture.	Total acidity in terms of lactic acid.	Volatile acidity in terms of acetic acid.	Nonvolatile acidity by difference.	Amino nitrogen.	Ammonia nitrogen.	Milligrams albuminoid nitrogen in 100 gm. dry silage.	Milligrams total nitrogen in 100 gm. dry silage.
1918.		*Per cent.*							
Sept. 9	0	89.0	447.6	.....	.....	69.1	18.0	77.0	125
10	1	71.84	671.4	.....	.....	86.3	14.28	.....	146
12	3	68.34	.....	.....	.....	.....	.....	.....	.....
13	4	66.2	2,293.95	208.8	2,085.15	124.5	17.0	.....	131
14	5	65.5	2,014.20	.....	.....	152.3	8.83	.....	157
16	7	65.0	2,629.65	268.5	2,362.15	176.1	13.92	.....	152
18	9	67.2	2,685.6	220.77	2,464.83	172.1	15.04	.....	133
20	11	66.4	2,831.07	313.23	2,517.84	149.1	5.96	.....	152
27	18	67.8	2,853.45	.....	.....	.....	.....	.....	.....
Oct. 18	39	69.7	2,942.97	.....	.....	176.1	.....	69.5	128

TABLE VI.—*Results of bacteriological examination of corn and soybean silage in concrete silo*

[Expressed in number of bacteria per gram]

Date sampled.	Number of days from ensiling.	Total in plain agar.	Total acid producers.		Colon group.		Bulgaricus group.	Yeast.	Liquefiers in gelatin.
			Dextrose litmus broth.	Cresol purple broth.	Bile lactose broth.	Lactose broth.			
1918.									
Sept. 9	0	45,000,000	10,000,000	1,000,000	1,000,000	10,000	28,500	10,000	1,000,000
10	1	220,000,000	100,000,000	100,000,000	10,000,000	1,000,000	58,000	1,000	1,000,000
12	3	500,000,000	1,000,000,000	1,000,000,000	10,000,000	100,000	630,000,000	10,000	10,000
13	4	1,100,000	10,000,000,000	10,000,000,000	100,000	1,000,000	25,000,000	10,000	100,000
14	5	400,000	100,000,000	100,000,000	100,000	1,000,000	60,000,000	10	1,000
16	7	610,000	1,000,000	1,000,000,000	1,000,000	100,000	28,000,000	1,000	100,000
18	9	.....	.....	100,000,000	10,000	10,000	6,400,000	100	.....
20	11	1,000,000	.....	100,000,000	10,000	10,000	16,000,000	.....	100
27	18	.....	.....	1,000,000,000	0	0	180,000,000	.....	1,000
Oct. 18	39	380,000	.....	10,000,000	100	100	29,000,000	.....	.....

TABLE VII.—*Results of chemical analysis of corn and soybean silage in concrete silo*

[Expressed in milligrams per 100 cc. of juice]

Date sampled.	Number of days from ensiling.	Moisture.	Total acidity in terms of lactic acid.	Volatile acidity in terms of acetic acid.	Nonvolatile acidity by difference.	Amino nitrogen.	Ammonia nitrogen.	Milligrams albuminoid nitrogen in 100 gm. dry silage.	Milligrams total nitrogen in 100 gm. dry silage.
1918.		*Per cent.*							
Sept. 9	0	69.0	525.9	.....	.....	52.4	12.92	75.0	133
10	1	71.34	760.9	.....	.....	74.2	8.72	.....	157
12	3	63.1	.....	.....	.....	.....	.....	.....	.....
13	4	62.9	1,846.3	229.7	1,616.6	120.7	10.88	.....	136
14	5	57.4	1,734.4	.....	.....	136.2	11.88	.....	134
16	7	62.2	2,036.5	480.2	1,556.3	195.6	10.52	.....	130.5
18	9	59.2	2,282.7	366.9	2,915.8	171.7	25.16	.....	138
20	11	62.7	2,327.5	322.1	2,005.4	191.4	20.4	.....	128
27	18	63.6	2,965.3	.....	.....	.....	.....	.....	.....
Oct. 18	39	66.0	2,998.9	384.8	2,614.1	216.4	17.0	67.5	119

It was thought that bacteriological and chemical results from silage treated as above might throw some additional light on the much debated

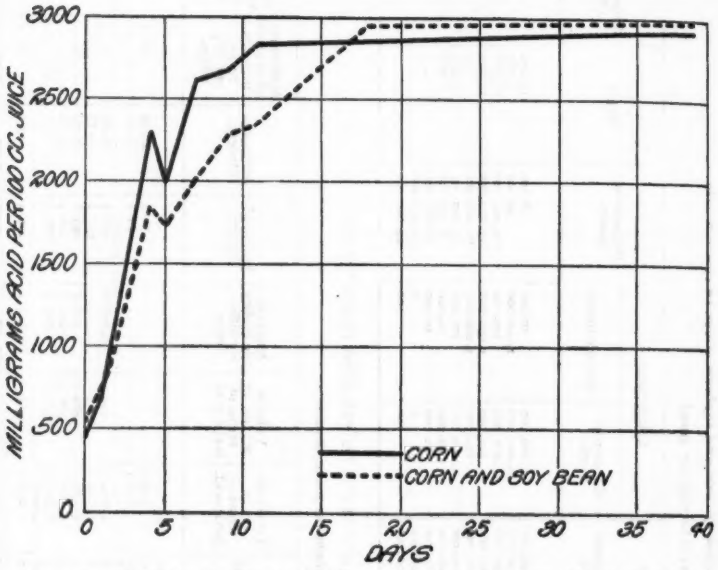


FIG. 1.—Graph showing development of acidity in corn silage and in corn and soybean silage.

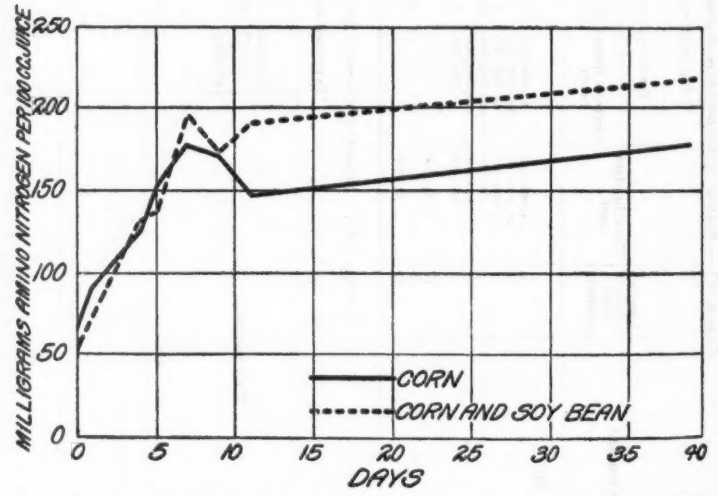


FIG. 2.—Graph showing development of amino nitrogen in corn silage and in corn and soybean silage.

question as to whether microorganisms or plant enzyms are the more important factors in silage fermentation.

EXPERIMENT IV

This experiment was performed at the same time and with the same crop as experiment I. This was done to compare the results of the large silo with a small laboratory silo. The material used in filling the

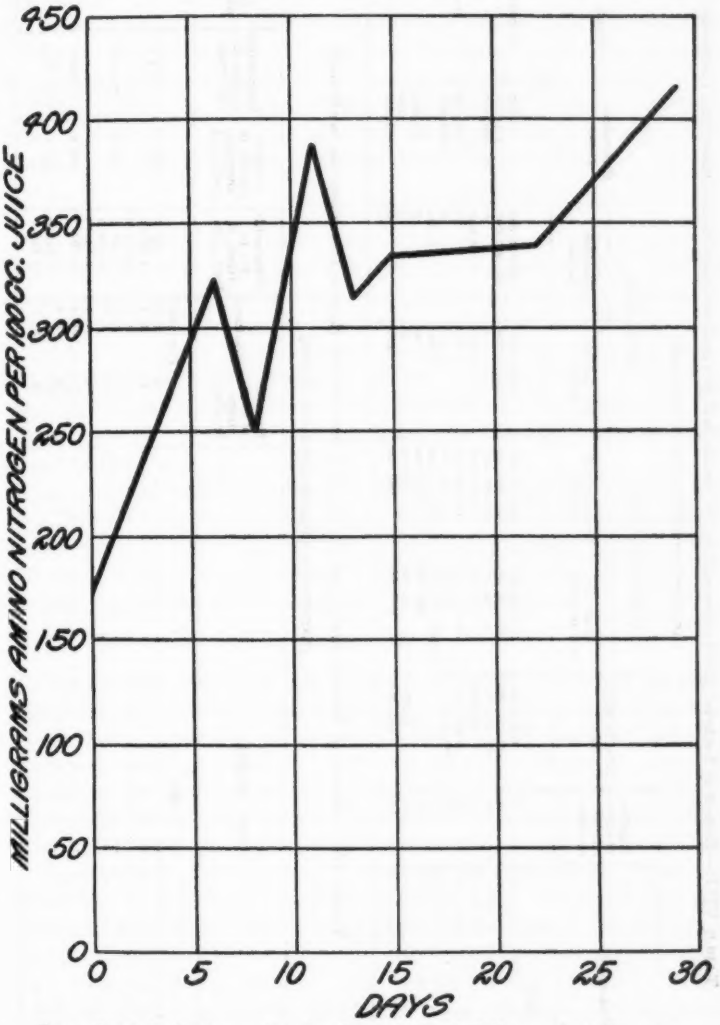


FIG. 3.—Graph showing increase in amino nitrogen in Canada field pea and oat silage, 1919.

laboratory silos was passed through a small grinder as it came from the silage cutter so as to make it sufficiently fine to be used in the small containers.

TABLE VIII.—*Results of bacteriological examination of Canada field pea and oat silage in laboratory silo (untreated)*

[Expressed in number of bacteria per gram]

Date sampled.	Number of days from ensiling.	Total in plain agar.	Total acid producers.		Colon group.		Bulgaricus group.	Yeast.	Liquefiers in gelatin.
			Dextrose litmus broth.	Cresol purple broth.	Bile lactose broth.	Lactose broth.			
1918.									
July 19	0	1,800,000	100,000,000	1,000,000,000	1,000,000	10,000,000	600	10,000,000	1,000
20	1	336,000,000	100,000,000	100,000,000	1,000,000	10,000,000	441,000	1,000	10
23	3	84,000,000	10,000,000	100,000,000	0	0	16,000,000	0	1,000
25	5	550,000	100,000	10,000,000	10,000	10,000	.....	10	10,000
27	7	9,900,000	10,000,000	10,000,000	1,000	10	109,000	10,000	1,000
30	10	5,500,000,000	100,000	1,000,000	1,000	0	1,000,000	10	0
Aug. 1	12	.....	10,000,000,000	1,000,000,000	1,000	1,000	.....	.....	.....
3	14	.....	100,000	1,000,000	0	0	282,000	0	100
7	18	55,000	10,000	10,000	0	0	750	0	100
14	25	180,000	100,000	100,000	100	0	138,000	0	0

TABLE IX.—*Results of chemical analysis of Canada field pea and oat silage in laboratory silo (untreated)*

[Expressed in milligrams per 100 cc. of juice]

Date sampled.	Number of days from ensiling.	Moisture.	Total acidity in terms of lactic acid.	Volatile acidity in terms of acetic acid.	Nonvolatile acidity, by difference.	Amino nitrogen.	Ammonia nitrogen.
1918.		*Per cent.*					
July 19	0	70.0	795.6	100.09	695.51	134.8	5.1
20	1	76.4	1,082.9	.....	.....	183.0	.....
23	3	85.2	2,873.0	.....	.....	261.0	60.84
25	5	76.0	2,541.5	350.3	2,191.2	213.1	55.76
27	7	76.8	2,986.3	.....	.....	238.1	.....
30	10	70.5	2,652.0	.....	.....	247.38	40.06
Aug. 1	12	61.4	2,762.5	500.5	2,262.0	251.2	62.80
3	14	73.2	.....	.....	.....	293.1	44.42
7	18	.....	2,818.0	571.1	2,246.9	307.3	49.98
14	25	87.1	2,828.0	.....	.....	279.6	.....

The bacteriological results given in Table VIII are characteristic of silage fermentation. The acid-producing organisms predominate. There was a variation in number of organisms belonging to the bulgaricus group. There were a large number of yeasts at the time of siloing, but a decided decrease occurred, and in a few days only a few were present. The number of gelatin liquefiers were few at time of storing and gradually decreased until there were no organisms belonging to this group after 25 days. Large numbers of organisms belonging to the colon group were present for the first day but then decreased rapidly.

Table IX gives the chemical results which show an increase in acidity, amino nitrogen, and ammonia as fermentation progresses. The greatest increase occurred during the first three days of fermentation.

EXPERIMENT V

The object of this experiment was to find out the effect of 2 per cent chloroform upon the bacterial flora and the chemical changes brought about by cell respiration. It was also desired to ascertain if silage so treated would undergo normal fermentation. (Tables X and XI.)

Table X gives the bacteriological results of Canada field pea silage treated with chloroform. From the results in this table it can be readily seen that chloroform was very efficient in inhibiting the growth of the microorganisms. After the first day there were few organisms belonging to any of the groups.

The chemical results are given in Table XI and show that there was very little increase in acidity. This indicates that the acidity in silage is due chiefly to microorganisms and is not due to plant enzyms. There was an increase in amino nitrogen and ammonia, showing that the hydrolysis of protein was principally due to cell respiration.

EXPERIMENT VI

Since an experiment had been planned to inhibit the growth of microorganisms, thus allowing cell respiration to play the principal part in fermentation, it was decided to perform one in which plant enzyms were destroyed, allowing action of only the microorganisms. A series of laboratory silos were filled with Canada field peas and oats and sterilized in autoclave for three hours. Each silo was then inoculated with fresh silage juice containing large numbers of microorganisms. (Tables XII and XIII.)

The bacteriological results with Canada field pea silage which was sterilized and inoculated are given in Table XII. They compare very favorably with those with normal silage, there being little difference. The first few days there was an increase in the total number of organisms, followed by a decrease. The acid-producing organisms constituted the main flora, and the number of these organisms decreased with the age of the silage. The number of organisms belonging to the colon group, yeast, and protein digesters decreased rapidly and after a few days disappeared. The number of organisms belonging to the bulgaricus group increased for the first five days and then gradually decreased.

TABLE X.—*Results of bacteriological examination of Canada field pea and oat silage in laboratory silo (treated with 2 per cent chloroform)*

[Expressed in number of bacteria per gram]

Date sampled.	Number of days from ensiling.	Total in plain agar.	Total acid producers.		Colon group.		Bulgaricus group	Yeast.	Liquefiers in gelatin.
			Dextrose litmus broth.	Cresol purple broth.	Bile lactose broth.	Lactose broth.			
1918.									
July 19	0	1,800,000	100,000,000	1,000,000,000	1,000,000	10,000,000	600	10,000,000	1,000
20	1	367,000	100,000,000	10,000,000	7,100,000	100,000	32,000	0	10
22	3	70,000	10,000	10,000	0	10	20	0	100
24	5	600	100	100	0	0	10	0	10
26	7	750	100	100	0	0	0	0	100
29	10	1,800	100	1,000	0	0	40	0	10
31	12	.....	10,000	1,000	100	71,000	.....	1,000	71,000
Aug. 2	14	700	100	100	0	10	.....	.....	100
6	18	900	10,000	100,000	0	0	6,180	0	1,000
13	25	800	100	100	0	0	45	0	100

TABLE XI.—*Results of chemical analysis of Canada field pea and oat silage in laboratory silo (treated with 2 per cent chloroform)*

[Expressed in milligrams per 100 cc. of juice]

Date sampled.	Number of days from ensiling.	Moisture.	Total acidity in terms of lactic acid.	Volatile acidity in terms of acetic acid.	Nonvolatile acidity, by difference.	Amino nitrogen.	Ammonia nitrogen.
1918.		*Per cent.*					
July 19	0	70.0	795.6	100.09	695.51	134.8	5.1
20	1	76	718.2	.....	.....	145.5	.....
22	3	79.4	663.0	.....	.....	197.5	10.54
24	5	73.6	751.4	.....	.....	231.2	51.00
26	7	75.3	663.0	53.04	609.96	216.9	42.5
29	10	70.8	773.5	.....	.....	221.3	42.7
31	12	69.9	497.2	255.6	241.6	203.8	44.2
Aug. 2	14	80.1	442.0	.....	.....	169.9	.....
6	18	74.4	773.5	147.3	626.5	236.0	37.25
13	25	86.5	895.0	.....	.....	269.5	38.76

TABLE XII.—*Results of bacteriological examination of Canada field pea and oat silage in laboratory silo (sterilized and inoculated)*

[Expressed in number of bacteria per gram]

Date sampled.	Number of days from ensiling.	Total in plain agar.	Total acid producers.		Colon group.		Bulgaricus group.	Yeast.	Liquefiers. in gelatin.
			Dextrose litmus broth.	Cresol purple broth.	Bile lactose.	Lactose broth.			
Inoculating juice		40,000,000	100,000,000	100,000,000	1,000,000	1,000,000	100	10,000	10
1918.									
July 20	1	345,000,000	1,000,000	1,000,000,000	1,000,000	1,000,000	46,000	0	0
22	3	310,000,000	1,000,000	10,000,000	0	0	250,000	0	0
24	5	270,000,000	100,000	10,000,000	0	0	18,000,000	0	0
26	7	1,400,000	1,000,000	10,000	0	0	5,440,000	0	0
29	10	1,500,000	10,000,000	10,000,000	0	10	.....	0	10,000
31	12	.....	10,000,000	10,000,000	10	10	.....	0	0
Aug. 2	14	520,000	71,000,000	71,000,000	0	0	.....	0	0
6	18	2,970,000	1,000,000	1,000,000	0	0	130,000	0	0
13	25	125,000	100,000	100,000	0	0	105	0	10

TABLE XIII.—*Results of chemical analysis of Canada field pea and oat silage in laboratory silo (sterilized and inoculated)*

[Expressed in milligrams per 100 cc. of juice]

Date sampled.	Number of days from ensiling.	Moisture.	Total acidity in terms of lactic acid.	Volatile acidity in terms of acetic acid.	Nonvolatile acidity by difference.	Amino nitrogen.	Ammonia nitrogen.
1918.		*Per cent.*					
July 19	0	70.0	795.6	100.9	695.51	38.4	5.1
20	1	77.0	828.7	.....	.....	57.4	.....
22	3	77.2	1,436.6	.....	.....	50.1	10.2
24	5	86.4	1,524.9	238.6	1,286.3	60.6	36.7
26	7	76.2	1,679.6	.....	.....	58.8	31.28
29	10	73.8	2,088.4	536.3	1,552.1	67.5	32.1
31	12	60.5	2,122.6	.....	.....	60.0	22.1
Aug. 2	14	.....	2,232.1	447.9	1,784.2	72.2	17.68
6	18	73.0	1,712.7	.....	.....	59.8	19.5
13	25	83.4	2,419.9	.....	.....	74.7	28.28

Inasmuch as microorganisms are the only agents concerned in the experiment, the results of the chemical analyses given in Table XIII show that the acidity was due chiefly to their activity. Part of the decomposition of protein with formation of ammonia was due to the action of microorganisms. Microorganisms played very little part in the formation of amino nitrogen.

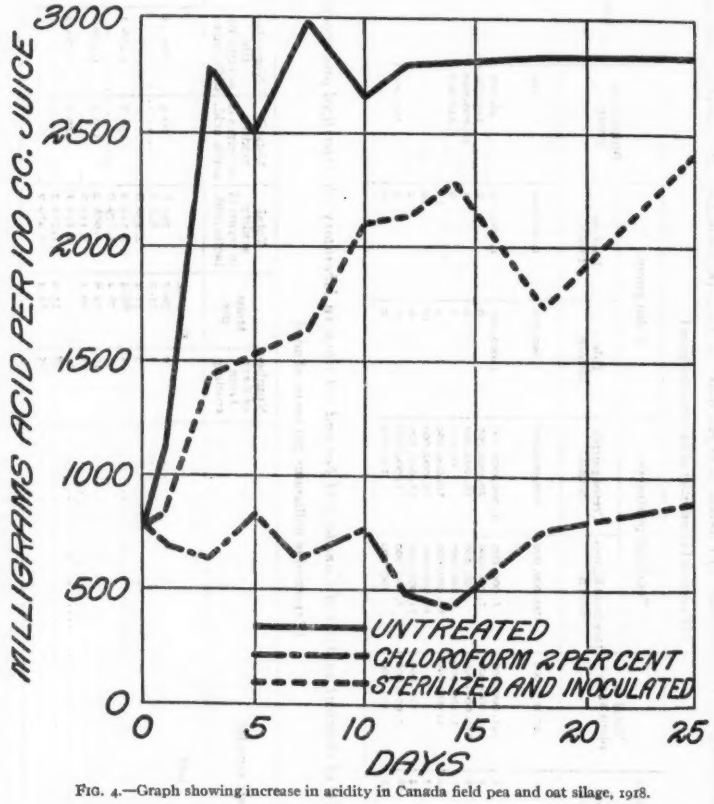


FIG. 4.—Graph showing increase in acidity in Canada field pea and oat silage, 1918.

In order that the increase in acidity, amino nitrogen, and ammonia in the laboratory experiments may be more easily compared, the results are given in figures 4, 5, and 6, respectively.

DISCUSSION OF RESULTS

The results of bacteriological and chemical analyses of various kinds of silage indicate that microorganisms are the principal agents in the fermentation of silage. It is quite natural to assume that enzyms would play some part in the fermentation, and it was found that enzyms did play a part in the hydrolysis of protein.

The production of acidity appears to be due almost entirely to micro-organisms. Silage treated with 2 per cent chloroform, thus inhibiting growth of bacteria, showed practically no increase in acidity, while silage sterilized and inoculated, which process destroyed plant enzyms,

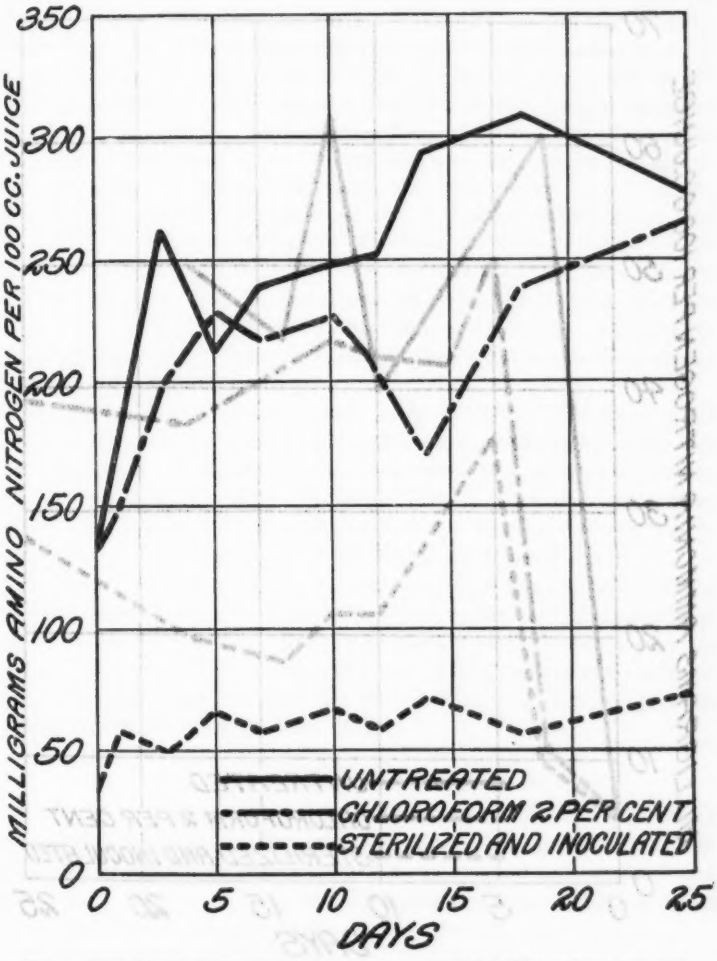


FIG. 5.—Graph showing development of amino nitrogen in Canada field pea and oat silage, 1918.

showed an increase in acidity closely resembling that of normal silage. The increase in the number of acid-producing organisms usually was followed by an increase in acidity.

The results of these investigations on the cause of formation of acid in silage agree with those of Lamb (*14*) and Hunter and Bushnell (*13*),

previously cited, in that the microorganisms are found responsible for the production of acid.

Plant enzyms evidently play an important part in the hydrolysis of protein, as is shown by the formation of ammonia after the silage had

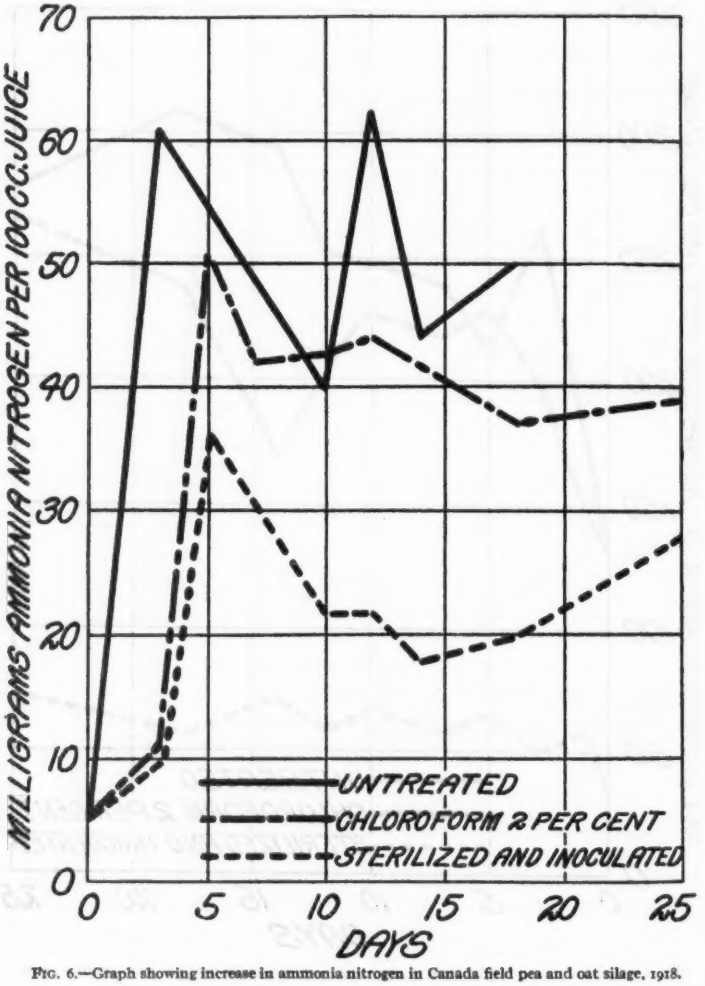


FIG. 6.—Graph showing increase in ammonia nitrogen in Canada field pea and oat silage, 1918.

been treated with 2 per cent chloroform, which inhibits the growth of bacteria. The formation of amino nitrogen in the chloroformed silage resembles that of normal silage. Practically no formation of amino nitrogen was observed in silage sterilized and inoculated. It is concluded that the hydrolysis of protein with formation of amino nitrogen

is due principally to the action of plant enzyms. Plant enzyms and microorganisms are both concerned in the formation of ammonia, the former being the more important.

The formation of ammonia occurred in silage treated with 2 per cent chloroform, which inhibits the growth of bacteria, and in silage sterilized and inoculated; but more ammonia was formed in the chloroformed silage than in that sterilized and inoculated. It will be noted that the increase in acid-producing organisms and the formation of ammonia are very well correlated. It is probable that the acid-producing organisms did attack the proteins, decomposing them with the formation of the ammonia. It has been shown by Bertrand and Weissweiller (*3*), Heineman and Hefferan (*10*), and Hastings, Evans, and Hart (*8*, *9*) that the lactic bacilli do have a digestive action on protein. Hart, Flint, and Evans (7), working on the action of certain bacteria in regard to the ripening of cheddar cheese, found that *Bacillus casei* had the ability to produce ammonia. Hunter (*12*) in an article on alfalfa silage suggests that a small part of the protein hydrolysis may be caused by the ability of acid-producing organisms to utilize protein as a source of energy in the absence of carbohydrates.

The largest number of yeasts were found in the first few days of the fermentation. They increased in number until all the free oxygen was consumed, then decreased. Lamb (*14*) states that alcohol is formed first by enzyms and later by yeasts. It is more easy to believe alcohol is formed first by yeasts and later by enzyms, because yeasts are present in greater numbers at the beginning of fermentation.

The only difference noted in the fermentation of Canada field pea and oat silage from that of corn silage was in the number of organisms belonging to the bulgaricus group. There were more organisms belonging to this group in corn silage than in silage composed of Canada field peas and oats.

No difference could be noted in the fermentation of corn silage when compared with corn and soybean silage.

CONCLUSIONS

(1) From the bacteriological and chemical analysis little difference can be noted between the fermentations taking place in silage composed of Canada field peas and oats, corn and soybeans, and corn only. There was a larger number of organisms belonging to the bulgarious group in corn silage than in the other types of silage studied.

(2) Production of acids was due to microorganisms.

(3) Yeasts apparently had little effect upon the fermentation of silage except during the first few days.

(4) Plant enzyms were chiefly responsible for the hydrolysis of protein with formation of amino nitrogen.

(5) The formation of ammonia was due to both enzyms and microorganisms.

LITERATURE CITED

(1) BABCOCK, S. M., and RUSSELL, H. L.
1900. CAUSES OPERATIVE IN THE PRODUCTION OF SILAGE. *In* Wis. Agr. Exp. Sta. 17th Ann. Rpt. [1899] 1900, p. 123–141, fig. 17.

(2) ——— ———
1901. CAUSES OPERATIVE IN THE FORMATION OF SILAGE. (SECOND PAPER.) Wis. Agr. Exp. Sta. 18th Ann. Rpt. [1900] 01, p. 177–184, fig. 44.

(3) BERTRAND, Gabriel, and WEISWEILLER, Gustav.
1907. EINWIRKUNG DES BULGARISCHEN FERMENTES AUF DIE MILCH. *In* Liebig's Ann. Chem., Bd. 351, p. 486–503.

(4) CONN, H. J., et al.
1918. METHODS OF PURE CULTURE STUDY. PRELIMINARY REPORT OF THE COMMITTEE ON THE CHART FOR IDENTIFICATION OF BACTERIAL SPECIES, [PRESENTED AT THE WASHINGTON MEETING OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS, DECEMBER 28, 1917]. *In* Jour. Bact., v. 3, no. 2, p. 115–128. References, p. 126.

(5) DOX, Arthur W., and NEIDIG, Ray E.
1912. THE VOLATILE ALIPHATIC ACIDS OF CORN SILAGE. Iowa Agr. Exp. Sta. Research Bul. 7, 32 p.

(6) ESTEN, W. M., and MASON, Christie J.
1912. SILAGE FERMENTATION. Conn. Storrs Agr. Exp. Sta. Bul. 70, 40 p. Bibliography, p. 37–40.

(7) HART, E. B., et al.
1914. RELATION OF THE ACTION OF CERTAIN BACTERIA TO THE RIPENING OF CHEESE OF THE CHEDDAR TYPE. *In* Jour. Agr. Research, v. 2, no. 3, p. 193–216. Literature cited, p. 214–216.

(8) HASTINGS, E. G., EVANS, Alice C., and HART, E. B.
1912. STUDIES ON THE FACTORS CONCERNED IN THE RIPENING OF CHEDDAR CHEESE. Wis. Agr. Exp. Sta. Research Bul. 25, 54 p., 6 fig.

(9) ——— ——— ———
1912. THE BACTERIOLOGY OF CHEDDAR CHEESE. U. S. Dept. Agr. Bur. Anim. Indus. Bul. 150, 52 p., 6 fig. Reprint of (8) above.

(10) HEINEMANN, P. G., and HEFFERAN, Mary.
1909. A STUDY OF BACILLUS BULGARICUS. *In* Jour. Infect. Diseases, v. 6, no. 3, p. 304–318, 5 fig.

(11) HUNTER, O. W.
1917. MICROORGANISMS AND HEAT PRODUCTION IN SILAGE FERMENTATION. *In* Jour. Agr. Research, v. 10, no. 2, p. 75–83, 10 fig. Literature cited, p. 82–83.

(12) ———
1918. BACTERIOLOGICAL STUDIES ON ALFALFA SILAGE. *In* Jour. Agr. Research, v. 15, no. 11, p. 571–592, 3 fig. Literature cited, p. 591–592.

(13) ——— and BUSHNELL, L. D.
1916. SOME IMPORTANT FERMENTATIONS IN SILAGE. Kans. Agr. Exp. Sta. Tech. Bul. 2, 32 p. Bibliography, p. 32.

(14) LAMB, A. R.
1917. THE RELATIVE INFLUENCE OF MICROORGANISMS AND PLANT ENZYMS ON THE FERMENTATION OF CORN SILAGE. *In* Jour. Agr. Research, v. 8, no. 10, p. 361–380, 13 fig. Literature cited, p. 378–380.

(15) ROUND, Lester A.
1917. THE BACTERIOLOGY OF SAUERKRAUT, A FURTHER STUDY. (Abstract.) *In* Abs. Bact., v. 1, no. 1, p. 50.

(16) RUSSELL, Edward J.
1908. THE CHEMICAL CHANGES TAKING PLACE DURING THE ENSILAGE OF MAIZE. *In* Jour. Agr. Sci., v. 2, pt. 4, p. 392–410.

(17) SAMARANI, Franco.
1913. STUDI INTORNO ALLA CONSERVAZIONE DEI FORAGGI ALLO STATO VERDE. *In* Bol. Min. Agr. Indus. e Com. [Rome], ser. C, anno 12, fasc. 8/12, p. 87–103.

(18) SHERMAN, James M.
1916. A CONTRIBUTION TO THE BACTERIOLOGY OF SILAGE. *In* Jour. Bact., v. 1, no. 4, p. 445–452. Bibliography, p. 452.

(19) ——— and BECHDEL, S. I.
1918. CORN-STOVER SILAGE. *In* Jour. Agr. Research, v. 12, no. 9, p. 589–600. Literature cited, p. 600.

(20) VAN SLYKE, Donald D.
1912. THE QUANTITATIVE DETERMINATION OF ALIPHATIC AMINO GROUPS. II. *In* Jour. Biol. Chem., v. 12, no. 2, p. 275–284, 1 tab., 2 fig., 1 pl. (fig. 1 on pl.).

SANDY CRYSTALS IN ICE CREAM: THEIR SEPARATION AND IDENTIFICATION

By HARPER F. ZOLLER, *Chemist*, and OWEN E. WILLIAMS, *Dairy Manufacturing Specialist, Dairy Division, Bureau of Animal Industry, United States Department of Agriculture*[1]

Ever since Sato (*6*)[2] isolated crystals of lactose, sucrose, calcium phosphate, calcium citrate, etc., from the crystalline sediment occurring in sweetened condensed milk, there has been considerable speculation as to which of these substances are responsible for most of the sandiness found therein.[3]

The ice-cream industry utilizes large quantities of condensed milk, particularly evaporated skim milk and sweetened condensed whole milk. The tendency during the last few years toward building up the total solids of the ice-cream mix with milk solids other than milk fat has created a demand for the products mentioned. With their use there arose simultaneously the problem of "sandy" ice cream. Not infrequently wholesale quantities of ice cream develop this sandy or crystalline texture—sometimes large, hard crystals, sometimes fine and gritty, like starch. This wide variation in the size of the crystals caused much perplexity as to their composition in view of the work cited above on condensed milk. Since ordinary ice cream contains from 12 to 16 per cent of sucrose it was but natural that many should believe the sandiness, especially the large crystals, to be due to this substance; the finer might be due to any of the suspected substances.

In order to settle this question of "what constitutes sandiness" we took up the problem of isolating the "sandiness" after it had formed and of identifying it.

DEVELOPMENT OF SANDY CRYSTALS

A large quantity of a high solids-not-fat ice cream was prepared in a regular commercial power freezer. The composition of the mix was as follows: Fat, 10 per cent; sucrose, 14 per cent; milk solids not fat, 12 per cent. The raw products were ripened 40 per cent cream, evaporated skim milk, and pure cane sugar. The mix was frozen in the regular time with brine at about 10° F. (− 12.22° C.). It was immediately packed, after freezing, in 2-quart tinned cans and placed in the hardening

[1] The authors express their indebtedness to G. L. Keenan and E. T. Wherry, of the Bureau of Chemistry, to the former for his courtesy in preparing the photomicrographs and to the latter for checking the crystallographic fea tures of lactose.

[2] Reference is made by number (italic) to "Literature cited," p. 795.

[3] It would be unfair to create the impression that Sato actually isolated sucrose crystals from all samples of condensed milk. In fact, the sucrose crystals were isolated only rarely from condensed milk, and in those cases he specifically states that they were condensed to an unusually low water content.

box. This box was insulated with wood and held a hardening mixture of ice and rock salt. The ice cream was repacked each day in this box.

On the fourth day very fine sandy crystals appeared. The crystals were developed by allowing the cans to become "heat shocked" (by setting them in contact with the air of the room for about 20 minutes) every other day after the sandiness first became perceptible.

The dimensions of the crystals were observed frequently through the low-power objective of a compound microscope. When the sandiness first became perceptible to the tongue and between the fingers, the crystals were clearly visible in the microscope. The general form of the crystals remained the same throughout 20 days' observation, although they grew much in size during this interval.

When the crystals had grown to a sufficient size they were separated from the parent mass. Plate 137 illustrates the appearance of the crystals in the frozen ice cream described above, when a drop of the ice cream was placed upon a glass slide and then viewed through the microscope in polarized light. The crystals showed plainly the natural maize-shaped form of lactose hydrate.

SEPARATION OF THE CRYSTALS

The sandy crystals were separated from several different preparations of ice cream by the following method: Frozen cream was allowed to melt at the temperature of the laboratory, about 25° C. When completely melted it was poured into large centrifugal tubes of 100 cc. capacity and centrifuged at 2,000 revolutions per minute for 10 minutes. The fat and liquid layers were poured from the sediment; the liquid portion was now entirely free from sandiness, the latter having been thrown down in the form of sediment. A drop of the sediment examined on the microscopic slide seemed to be made up entirely of crystals of one form—some large and some small.

Before the crystals were proved to consist entirely of pure lactose they were isolated from the sediment for the purpose of holding any sucrose or lactose crystals in the crystalline form. This was effected by saturating water with all the sucrose and lactose that it could hold at 2° C. It was then warmed to 5°, and the sediment in each tube was thoroughly shaken with about 75 cc. of it. Then the tubes were again centrifuged. This operation was repeated with the addition of 50 per cent acetone to the washing fluid. The product was further freed from liquid on a Büchner funnel.

The crystals were obtained in a pure white condition. More than ½ kilo was obtained in this manner. They were dried to constant weight on filter paper in a boiling-water oven. Plate 138, A, illustrates the appearance of these isolated crystals. They show plainly the effect of the washing mixtures but retain their characteristic maizelike or wedge-like form.

IDENTIFICATION OF THE CRYSTALS AS LACTOSE HYDRATE

MICROSCOPICAL EXAMINATION

The ease with which milk sugar usually crystallizes and the very characteristic form of its crystals suggest the use of the microscope as the most accurate means of identifying it. As mentioned in the earlier part of the text it is possible to detect and follow microscopically the growth of the crystals in the ice cream.

For the purpose of identification of the sandy crystals it was necessary to have at hand pure specimens of lactose and sucrose crystals. Rather than accept the market lactose as standard for recrystallization a quantity of pure lactose was obtained by evaporating some milk whey from cheese-making in a vacuum pan to a concentration of 8 parts by weight of whey to 1 part by weight of product. The resulting lactose was filtered and purified according to the customary methods.

The crystalline lactose thus obtained was the α form, or commonly recognized lactose hydrate ($C_{12}H_{22}O_{11}.H_2O$). According to Groth (*1, p. 450*) its crystallographic features are—

Monoclinic-sphenoidal. Cleavage in three directions nearly at right angles. Refractive indices, $\alpha = 1.517$; $\beta = 1.542$; $\gamma = 1.550 \pm 0.005$ $Bx^a \wedge c = 10°$, $\wedge a = 99°$. $2E = 33\text{-}1/2°$. Sign $-$, sp. gr. $1.525 - 1.534$.

Some of these crystals were placed upon a microscopic slide, and a photomicrograph was obtained with polarized light. The photograph exhibits the characteristic tomahawk-shaped crystals which are always presented by α-lactose when the crystals are allowed to develop spontaneously below 93° C. (*3*). When they crystallize at 93°, or a little above, lenticular needles appear (γ or anhydride form) (*2*), which are markedly different to the eye. These gradually transform into the tomahawk type as the temperature drops below 93°. The photomicrograph of these lactose-hydrate crystals is shown in Plate 138, B.

The comparison of these pure lactose crystals with those within the ice cream in Plate 137, as well as with that of the sandy crystals separated in a pure condition from the same ice cream, shown in Plate 138, A, shows the sandy crystals to be identical in form with those of pure lactose. It is but natural that we should realize the effects shown in Plates 137 and 138, A, of substances tending to hinder perfect growth of the crystals. Also the solvent action of water is evident in the partially dissolved facets and less sharp outlines. Otherwise there is no difference in the appearance between the crystals in Plate 138, B, and those in the first three figures.

A fully developed crystal of sucrose is shown in Plate 138, C. The sucrose crystals were grown within a pectin gel from a supersaturated sucrose solution (500 per cent solution) at ordinary temperature. There is not the least resemblance between this crystal and those isolated from ice cream.

CHEMICAL ANALYSIS

Some of the isolated sandy crystals mentioned in the section on separation, and similar to those photographed in Plate 138, A, were analyzed for lactose, without inversion, according to the Munson and Walker method,[1] described by Leach (*4, p. 598*). The average of three determinations gave 99.86 per cent calculated as lactose hydrate from Munson and Walker's tables. It is evident that the quantity of sucrose appearing as sandy crystals is insignificant.

DISCUSSION

The results of our experiments upon a number of sandy ice creams lead us to conclude definitely that the sandiness is due to the crystallization of lactose from a supersaturated solution.

The suspicion, fostered by a number of persons in the dairy industry, that sucrose is responsible for this sandiness is not founded on scientific principles. The solubility, recorded by many investigations, of lactose in water at 10° C. is about 17 gm. per 100 gm. of water. The solubility of sucrose at 12° is given as 198.6 gm. per 100 gm. of water. When these solutions are cooled they become supersaturated, provided they contain the above-mentioned quantities of the respective sugars. In sucrose solutions, however, we meet with the experience of the sugar technician, Prinsen Geerligs (*5, p. 301*), who tells us that sucrose solutions frequently act like glue at low temperatures and can be supercooled to a state in which they can not be poured. In the highly supersaturated, supercooled condition it is very difficult to induce them to crystallize. When accomplished it requires weeks and even months for the crystals to develop in size, particularly if much colloidal material, such as gum, pectin, or protein, is present in the solution. It required several months for the perfect crystal of sucrose shown in Plate 138, C, to develop even to this size.

In the case of lactose, because of its low solubility in water, compared to sucrose, it is more difficult to form supersaturated solutions of any great magnitude. When its solutions are concentrated it crystallizes without much excitation. Indeed, it will persist in crystallizing even in the presence of complex protective substances which check crystallization of many other compounds.

An ice-cream mix of 10 per cent fat, 12 per cent milk solids not fat, and 14 per cent sucrose contains, if we assume that the two sugars compete for only the water in the mix, about 22 per cent sucrose solution and about 10.5 per cent lactose solution. When we recall the fact that sucrose is about 10 times as soluble as lactose in water, we readily see that the physical conditions existing in frozen ice cream are unsuited to the crystallization of sucrose.

[1] MUNSON, L. S., and WALKER, Percy H. UNIFICATION OF REDUCING SUGAR METHODS. *In* Jour. Amer. Chem. Soc., v. 28, no. 6, p. 663–686, 1906; v. 29, no 4, p. 541–554, 1907; Final tables *in* U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), p. 241–251, 1908; A correction, U. S. Dept. Agr. Bur. Chem. Circ. 82, 6 p., 1911.

SUMMARY

(1) The "sandiness" in ice cream has been isolated and identified as lactose.

(2) The growth of the "sand" crystals can be followed satisfactorily with the microscope.

(3) The form of lactose appearing in the ice cream is the normal α crystal which crystallizes from water solutions in tomahawk-shaped prisms and from protein solutions in more rugged or maize-shaped crystals.

LITERATURE CITED

(1) GROTH, P.
1910. CHEMISCHE KRYSTALLOGRAPHIE. TEIL 3. ALPHABETISCHE UND HYDROAROMATISCHE KOHLENSTOFFVERBINDUNGEN. iv, 804 p., fig. 912–1559. Leipzig. Bibliographical footnotes.

(2) HUDSON, C. S.
1904. THE HYDRATION OF MILK-SUGAR IN SOLUTION. *In* Jour. Amer. Chem. Soc., v. 26. no. 9, p. 1065–1082.

(3) ———
1908. FURTHER STUDIES ON THE FORMS OF MILK-SUGAR. *In* Jour. Amer. Chem. Soc., v. 30, no. 11, p. 1767–1783, 2 fig.

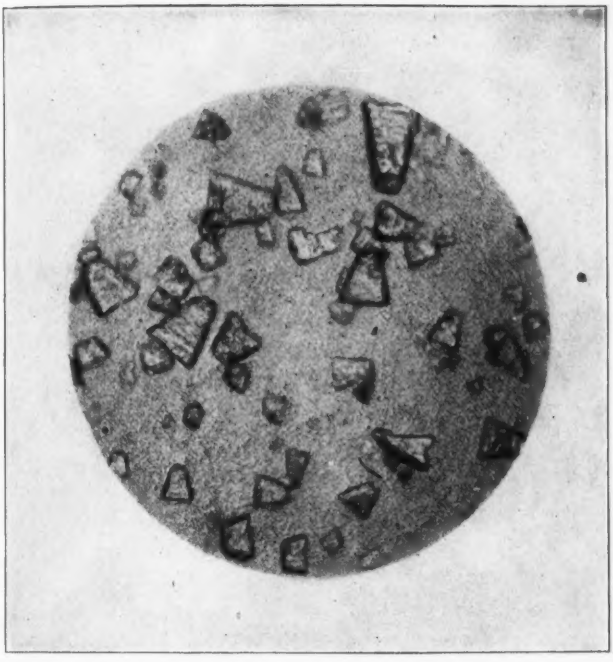
(4) LEACH, Albert E.
1913. FOOD INSPECTION AND ANALYSIS ... REV. AND ENL. BY ANDREW L. WINTON ... ed. 3, xix, 1001 p., 120 fig., 40 pl. New York, 1913. Bibliographies at ends of chapters.

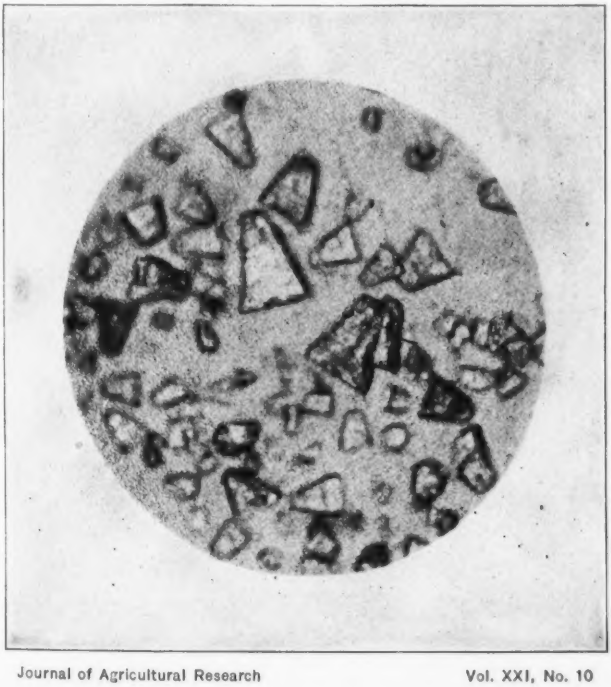
(5) PRINSEN GEERLIGS, H. C.
1909. CANE SUGAR AND ITS MANUFACTURE. xi, 350, xvi p. Altrincham, England.

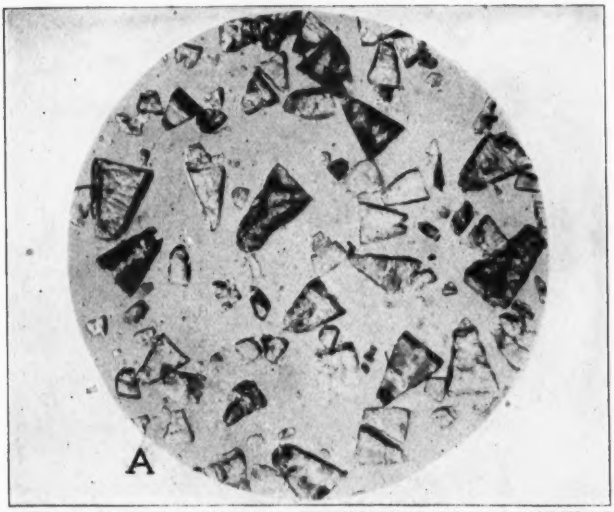
(6) SATO, M.
1914. UNTERSUCHUNGEN ÜBER DIE MILCHKRYSTALLE IN KONDENSIERTER MILCH MIT ZUCKERZUSATZ. *In* Jour. Col Agr. Tohoku Imp. Univ., v. 5, pt. 9, p. 321–338, pl. 11–18.

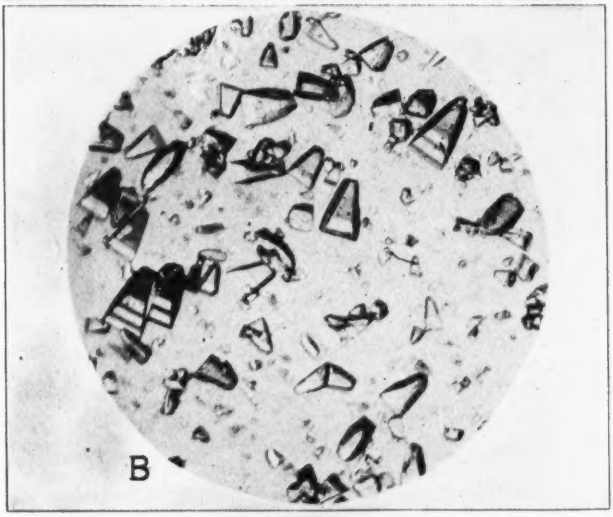
PLATE 137

Photomicrographs of the "sand" crystals in ice cream, made by placing a drop of the melted ice cream upon a microscopic slide and covering it with a cover glass. This was then placed upon the stage of the photographic microscope and photographed with polarized light. × 90.






A


B

C

PLATE 138

A.—Photomicrograph of the isolated "sand" crystals from some of the same ice cream used for Plate 137. Photographed with polarized light. × 90.

B.—Photomicrograph of some pure recrystallized lactose hydrate from condensed whey. Photographed with polarized light. × 90.

C.—Photomicrograph of a crystal of pure sucrose. Photographed in plane light. × 5.